Medical Genetics

Volume IV
Therapeutic Genetics

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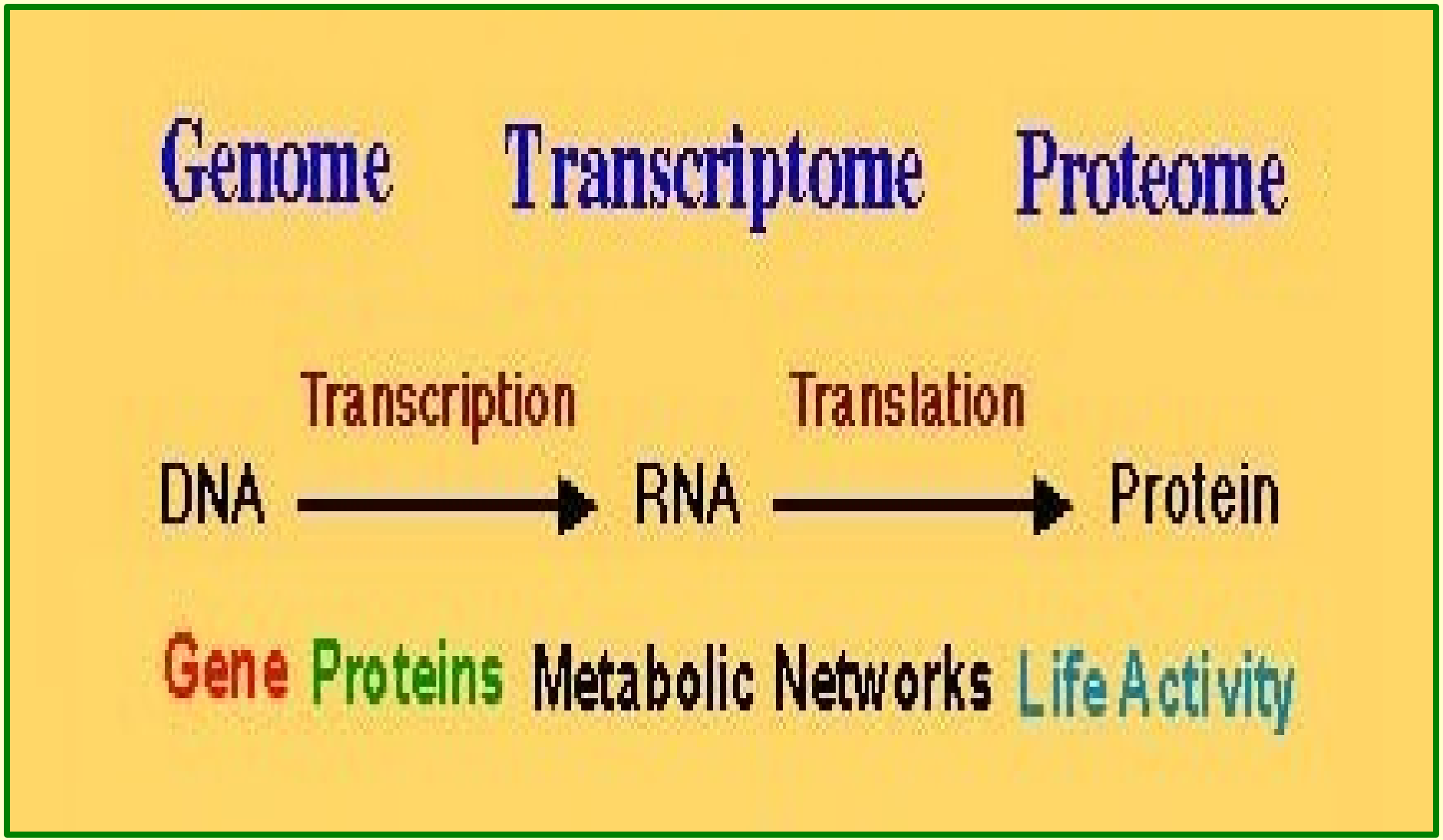
Spectrum of Medical Genetics

Basic Genetics	Clinical Genetics
Part I: Molecular Genetics Part II: Biochemical Genetics Part III: Physiological Genetics Part IIII: Cytogenetics Part V: Pathogenetics Part VI: Pharmacogenetics Part VII: Oncogenetics Part VIII: Immunogenetics Part IX: Formal Genetics Part X: Population genetics Part XI: Developmental Genetics Part XII: Genomics Part XIII: Transcriptomics Part XIV: Proteomics	Part I: Chromosomal Aberrations Part II: Congenital Malformations Part III: Inborn Errors of Metabolism Part IV: Mitochondrial Disorders Part V: Genetic Systemic Syndrome Part VI: Genetic Diseases of The Nervous system Part VII: Genetic Diseases of The Endocrinal system Part VIII: Genetic Diseases of The Cardio-Vascular system Part IX: Genetic Diseases of The Respiratory system Part X: Genetic Diseases of The Gastro-Intestinal system Part XI: Genetic Diseases of The Urinary system Part XII: Genetic Diseases of The Muscular system Part XIII: Genetic Diseases of The Skeletal system Part XIV: Genetic Diseases of The Blood system Part XV: Genetic Diseases of The Immunity system Part XVI: Genetic Diseases of The Male Genital system Part XVII: Genetic Diseases of The Female Genital system Part XVIII: Genetic Diseases of The Ocular system Part XIX: Genetic Diseases of The Auditory system Part XX: Genetic Diseases of The Skin Part XXI: Genetic Psychiatric Disorders
Diagnostic Genetics	**Therapeutic Genetics**
Part I: Molecular Diagnostic Techniques Part II: Cytogenetic Diagnostic Techniques Part III: Biochemical Diagnostic techniques Part IV: Prenatal Diagnosis Part V: Pre-Implantation Diagnosis Part VI: Per-Symptomatic Diagnosis Part VII: Conventional Diagnostic Techniques	Part I: Pharmacologic Therapy Part II: Nutritional Therapy Part III: Replacement Therapy Part IV: Transplantation Therapy Part V: Stem Cell Therapy Part VI: Surgical Intervention Part VII: Genetic Therapy Part VIII: Fetal Therapy Part IX: Conventional Therapy
Prophylactic Genetics	**Applied Genetics**
Part I: Pre-Conception Prophylaxis Part II: Pre-Natal Prophylaxis Part III: Pre-Symptomatic Prophylaxis	Part I: Forensic Genetics Part II: Genetic Counseling Part III: Genetic Screening Part IV: Genetic Engineering Part V: Eugenics

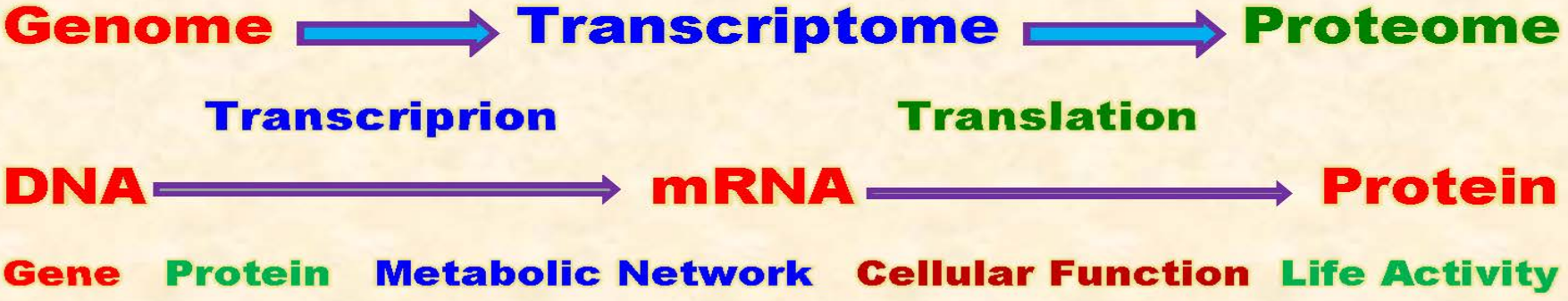
Dogma of Molecular Biology

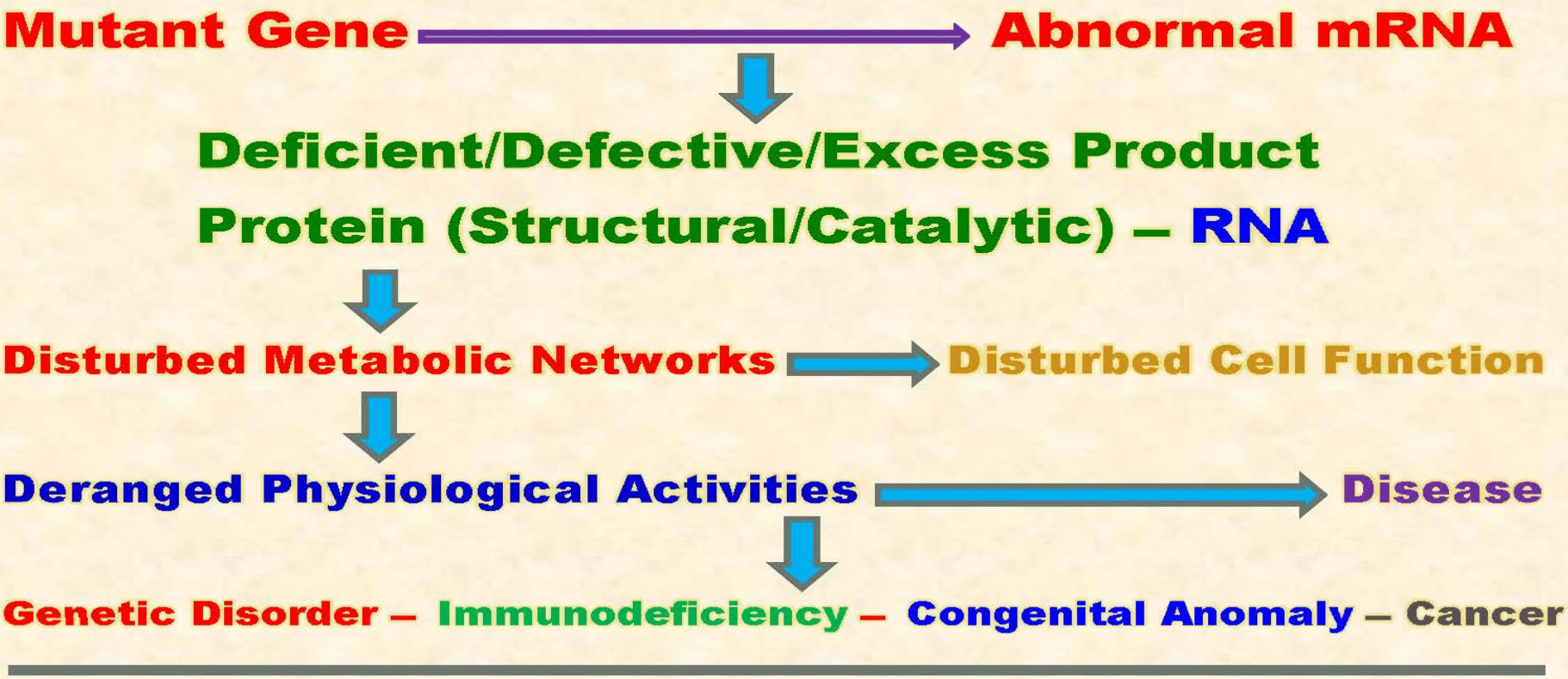
Relation Between The genetic Material and Life Activities

Life Activities at The Molecular level



Dogma Of Molecular Pathology In Health And Disease





Therapeutic Approaches To Genetic Diseases

1. Damaging Substrate Restriction Approach.
2. Toxic Substrate Removal Approach.
3. Deficient Substrate Replacement Approach.
4. Bioactive Molecules Supplementary Therapy
5. Substrate Reduction Therapy.
6. Pharmacologic Drug Therapy.
7. Metabolic Manipulations
8. Organ, Tissue, Cell Transplantation.
9. Surgical Intervention.
10. Fetal Therapy.
11. Genetic Therapies

Therapeutic Approaches To Genetic Diseases

1. Damaging Substrate Restriction Approach

A nutritional or dietary management approach.

A. Protein Restriction Therapy

Restriction of Protein intake in PKU, Homocystinuria, Urea Cycle Defect, MSUD.

B. Carbohydrate Restriction Therapy

Galactosemia, Hereditary Fructose intolerance, Diabetes Mellitus.

C. Lipid Restriction Therapy

Hypertriglyceridemia: Reducing saturated versus poly- and monounsaturated fats, Increased consumption of Omega-3 fatty acids.

Genetic Disorders amenable to Dietary Restrictions/Managements

Disorder	Example
Disorders of Amino acids Metabolism	1- Phenylketonuria 2- Urea cycle defects 3- Maple syrup urine disease 4- Tyrosinemia type I
Disorders of Carbohydrate Metabolism	1. Diabetes mellitus 2. Galactosemia
Disorders of Organic acids metabolism	1- Glutaric academia type I 2- Methylmalonic academia 3- Isovaleric academia
Dietary Restriction in Hyperlipidemias	1- Familial hypercholesterolemia 2- Familial hypertriglyceridemia 3- Familial combined hyperlipidemia 4- Familial hyperchylomicronemia
Dietary Restriction in Heavy metal Disorders	1- Iron restriction in Hemochromatosis, Thalassemia. 2- Copper restriction in Wilson disease 3. Manganese restriction in Hypermagnesemia with dystonia.
Disorders of Purine metabolism	Gout
Dietary Restriction in Celiac disease	

2. Toxic Substrate Removal Approach

A. Hemodialysis/Peritoneal Dialysis: Hyperammonemia.

B. Mineral Chelation Therapy

1. Iron Chelation (Deferoxamine, Deferasirox)

Thalassemia, Hereditary Hemochromatosis, Myelo-dysplasic Syndromes, Acaeruloplasminaemia, Cerebellar ataxia, Neuroferritinopathy, Hyperferritinemia-cataract syndrome, L-ferritin deficiency, Ponto-cerebellar hypoplasia.

2. Cupper Chelation (Penicillamine, Trientine, Dimercaprol)

Wilson Disease, Alzheimer Disease, Parkinson Disease, Idiopathic Pulmonary Fibrosis, DM.

3. Manganese Chelation (Para-Aminosalicylic Acid)

Manganese (Mn)-Induced Parkinsonism, Hypermagnesemia with Dystonia.

Disorders that are due to an accumulation of manganese, copper, or iron and cause neurotoxicity.

Disorders	Transition Metal	Inheritance	Gene	OMIM	Gene Function	Symptoms
Hypermanganesemia with dystonia 1	Manganese	Autosomal recessive	*SLC30A10*	613280	Manganese transporter	Dystonia, cock-walk gait Parkinsonism
Hypermanganesemia with dystonia 2	Manganese	Autosomal recessive	*SLC39A14*	617013	Manganese transporter	Progressive dystonia and bulbar dysfunction
Wilson's disease	Copper	Autosomal recessive	*ATP7B*	277900	Copper transporter	Dysarthria, dysphagia, tremor, dystonic rigidity
Acaeruloplasminaemia, Cerebellar ataxia, Hypoceruloplasminemia	Iron	Autosomal recessive	*CP*	604290	Ferroxidase	Chorea, ataxia, dystonia, Parkinsonism, Diabetes mellitus
Neuroferritinopathy, Hyperferritinemia-cataract syndrome, L-ferritin deficiency	Iron	Autosomal dominant	*FTL*	600886 615604 606159	Iron storage	Chorea, dystonia
Spastic paraplegia type 35	Iron	Autosomal recessive	*FA2H*	612319	Fatty acid 2-hydroxylase (Synthesis of sphingolipids)	Gait difficulties with spastic paraparesis and dysmetria
Neurodegeneration with brain iron accumulation 1, HARP syndrome	Iron	Autosomal recessive	*PANK2*	607236 234200	Pantothenate kinase (CoA synthesis)	Dystonia, rigidity, choreoathetosis
Neurodegeneration with brain iron accumulation 6, Pontocerebellar hypoplasia type 12	Iron	Autosomal recessive	*COASY*	615643 618266	CoA synthesis	Dystonia, rigidity, choreoathetosis
Infantile neuroaxonal dystrophy 1, Neurodegeneration with brain iron accumulation 2B. Parkinson's	Iron	Autosomal recessive	*PLA2G6*	256600 610217	Phospholipase	Motor regression, hypotonia

3. Deficient Substrate Replacement Approach

A. Protein Replacement Therapy

Anti-hemophilic Globulin: Hemophilia, Immunoglobulins: Immunodeficiency.

B. Enzyme Replacement Therapy (ERT)

Fabry disease : Aglsidase Alfa and Agalsidase Beta.

Pompe Disease : Alglucosidase Alpha.

Type 1 Gaucher Disease : Cerezyme(Imiglucerase)

Cystic Fibrosis: Recombinant DNase

MPS type II (Hunter syndrome): Idursulfase.

MPS type IVA (Morquio A syndrome): Elosulfase Alfa.

MPS type VI (Maroteaux-Lamy syndrome): Recombinant Human N-acetylgalactosamine 4-sulfatase.

Severe Combined Immune Deficiency (SCID): Adenosine Deaminase.

C. Hormone Replacement Therapy

L-Thyroxine: Hypothyroidism.

GH: Growth Hormone Deficiency.

Corticosteroids: Addison Disease.

Erythropoietin: Renal-Failure Induced Anemia.

Examples of Genetic Diseases Treated Via Addition-Replacement Approach

Protein Replacement Therapy	Hormone Replacement Therapy	Enzyme Replacement Therapy
Hemophilia A (Factor VIII-Anti-hemophilic Globulin)	**Growth Hormone deficiency** (Growth Hormone)	**Gaucher disease Type 1** (Recombinant β-Gluosidase).
Hemophilia B (Factor IX)	**Hypothyroidism** (Thyroxine)	**Fabry disease** (Recombinant α-Galatosidase A).
Multiple sclerosis (Interferon β-1 A, Interferon β-1 B)	**Insulin Dependent Diabetes Mellitus** (Insulin)	**Pompe disease** (Recombinant α-Glucosidase)
Humoral immunodeficiency (Immunoglobulins)	**Dyserythropoietic Anemia** (Erythropoietin)	**Cystic fibrosis** (Recombinant Inhaled DNase & Pancreatic Enzyme Preparations).
	Suprarenal Deficiency Disorders (Corticosteroids)	**Phenylketonuria** (Phenylalanine Ammonia Lyase)
	Gonadotrophin Deficiency States (Gonadotrophin)	**α1-Antytrypsin Deficiency** (α_1-Proteinase Inhibitor).
	Ostoeporosis (Parathormone)	**Enterokinase Deficiency** (Pancreatic Enzymes)
	Lipodystrophy, Leptin Deficiency (Leptin)	**Adenosine Deaminase Deficiency** (Recombinant Adenosine Deaminase)
		Hurler/Hurler-Schie Syndrome (Mucopolysaccharidosis Type I) (Recombinant Human α-L-iduronidase).

4. Bioactive Molecules Supplementary Therapy

A. Megadose Vitamin Therapy

B6-Dependent Homocystinuria.

B12-Dependent Congenital pernicious anemia.

Folic Acid: Homocystinuria, Megaloblastic Anemia.

B. L-Carnitine

Mitochondrial Disorders, Organic Acidurias, Fatty Acid Oxidation Defects.

C. Tri-methyl Glycine (Betaine) (Methyl Donor)

Homocystinuria (Converts Homocysteine to Methionine).

D. Zinc Acetate

Wilson Disease (Decreases Cupper Absorption).

E. Trace Minerals

Selenium: Hashimoto disease, Graves disease, Immunodeficiency.

Zinc: Acrodermatitis enteropathica.

Cupper: Menkes Disease.

Iron: Iron Refractory Iron Deficiency Anemia.

F. Gentamycin & Streptomycin (Gene-Editing Molecules)

Cystic Fibrosis, Hurler syndrome.

G. Melatonin

Cancer, Oxidative Stress, Radiation-Induced Clastogenesis

Due to its DNA protective effects.

5. Substrate Reduction Therapy (SRT)

The general principle of SRT, is that a small molecule drug may be used to partially inhibit the biosynthesis of the compounds, which accumulate in the absence of a specific lysosomal enzyme. By doing so, the drug reduces the number of molecules requiring catabolism within the lysosome, thus contributing to balance the rate of synthesis with the impaired rate of catabolism. Theoretically, this approach had a number of potential advantages when compared with ERT, including oral availability, non-immunogenicity, the use of a single compound to treat a number of diseases as well as the possibility of being able to reduce storage in the brain.

Miglustat is a small imino-sugar molecule that acts as a competitive inhibitor of the enzyme glucosylceramide synthase, which catalyzes the first committed step in glycosphingolipid (GSL) synthesis, the glycosylation of ceramide. It is only used for patients who cannot be treated with enzyme replacement therapy with Imiglucerase.

Miglustat is now the first and only approved therapy for patients with Niemann-Pick disease type C1 (NP-C).

Therapeutic Applications of Substrate Reduction Therapy Approach In Storage Disorders

1. Type 1 Gaucher Disease: Glucocerebrosidase and SRT (Miglustat).

2. Sandhoff Disease: SRT (Miglustat) with a ketogenic diet.

3. Niemann-Pick type C1: SRT (Miglustat) with Curcumin and Ibuprofen.

Miglustat targets sphingolipid synthesis and storage, while Curcumin compensates lysosomal Calcium defect, and Ibuprofen reduces CNS inflammation.

Triple combination therapy proved to have greater neuro-protective benefits than mono- or dual-therapy.

6. Pharmacologic Drug Therapies

Hydroxy Urea & Piracetam: Sickle Cell Anemia.

Hydroxychloroquine & Hematin: Porphyria.

Chemotherapeutics: Cancer.

Anti-Epileptic Drugs: Epilepsy.

Lipid Lowering Drugs: Hyperlipidemia

7. Metabolic Manipulations

A. Metabolic Activation

Use of Phenobarbital to increase the activity of glucoronyl-transferase enzyme to enhance hepatic metabolism of bilirubin and reduce its level in states of hyper-bilirubinemia like Gilbert syndrome and Crigler-Najjar syndromes.

B. Metabolic Inhibition

1. Direct inhibition

Use of the inhibitory effect of Nitisinone (NTBC) on the enzyme 4-hydroxyphenylpyruvate dioxygenase as a complementary step in management of tyrosinemia type I. NTCB prevents the formation of fumaryl-acetoacetate from tyrosine and blocks the accumulation of the toxic metabolites thus reducing their toxic effects on body organs and tissues.

2. Competitive inhibition

Use of the inhibitory effect of Statins on the function of the Hydroxymethylglutaryl-CoA reductase enzyme leading to reduction in synthesis of cholesterol and lowering of serum LDL cholesterol concentrations.

8. Organ, Tissue, Cell Transplantation Therapy

A. Organ Transplantation

Kidney, Liver, Heart, Lung, Pancreas, Bone Marrow Transplantation.

B. Tissue Transplantation

Cornea Transplantation, Heart' Valve Transplantation, Bone Grafting.

C. Cell Transplantation

Whole/Fractionated Blood Transfusion, Myoblast Transplantation, Hepatocyte Transplantation.

9. Surgical Intervention

A. Structural Congenital Malformations and late developing genetically-determined malformations.

B. Some genetic metabolic disorders: e.g. jejuno-ileal by-pass, end-to-side porta-caval shunt, and partial ileal by-pass for some morbid genetic hyperlipidemia and dyslipidemia states.

10. Fetal Therapy

A. Fetal Drug Therapy

Corticosteroids: 21-Hydroxylase Deficiency (Congenital Adrenal Hyperplasia).

L-Thyroxine: Fetal Hypothyroidism.

B12: B12-Responsive Fetal Methyl-malonic Acidemia.

Biotin: Biotin-Responsive Fetal Multiple Carboxylase Deficiency.

B. Fetal Surgical Intervention

Fetal Hydronephrosis, Fetal Hydrocephalus, Fetal Diaphragmatic Hernia, Fetal Heart Anomalies.

11. Genetic Therapies

A. Whole Genome Therapy

Stem Cell Therapy

1. Embryonic stem cell therapy

2. Adult stem cell therapy

B. Gene Therapy

1. Delivery of Normal Genes to Compensate for Mutant Genes.

2. Repair of Mutant Genes.

3. Disruption of Over-expressing Genes in Cancer Cells.

C. m-RNA Therapy

Interfering complementary m-RNA in Cancer Cells.

mRNA Transfer-replacement.

mRNA Editing (Nusinersen in SMA).

D. micro-RNA Therapy

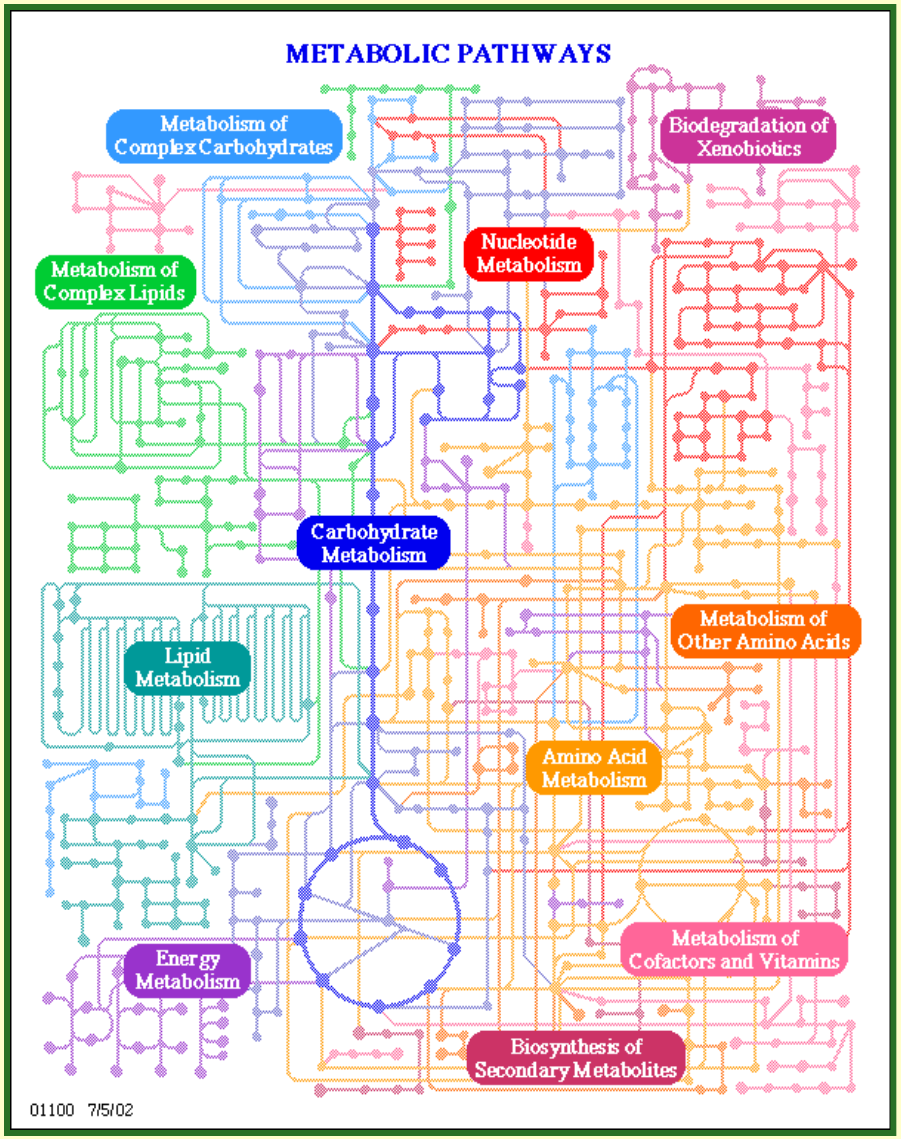
piwi-RNA: Prophylaxis of congenital malformations caused by transposon hyperactivity during pregnancy.

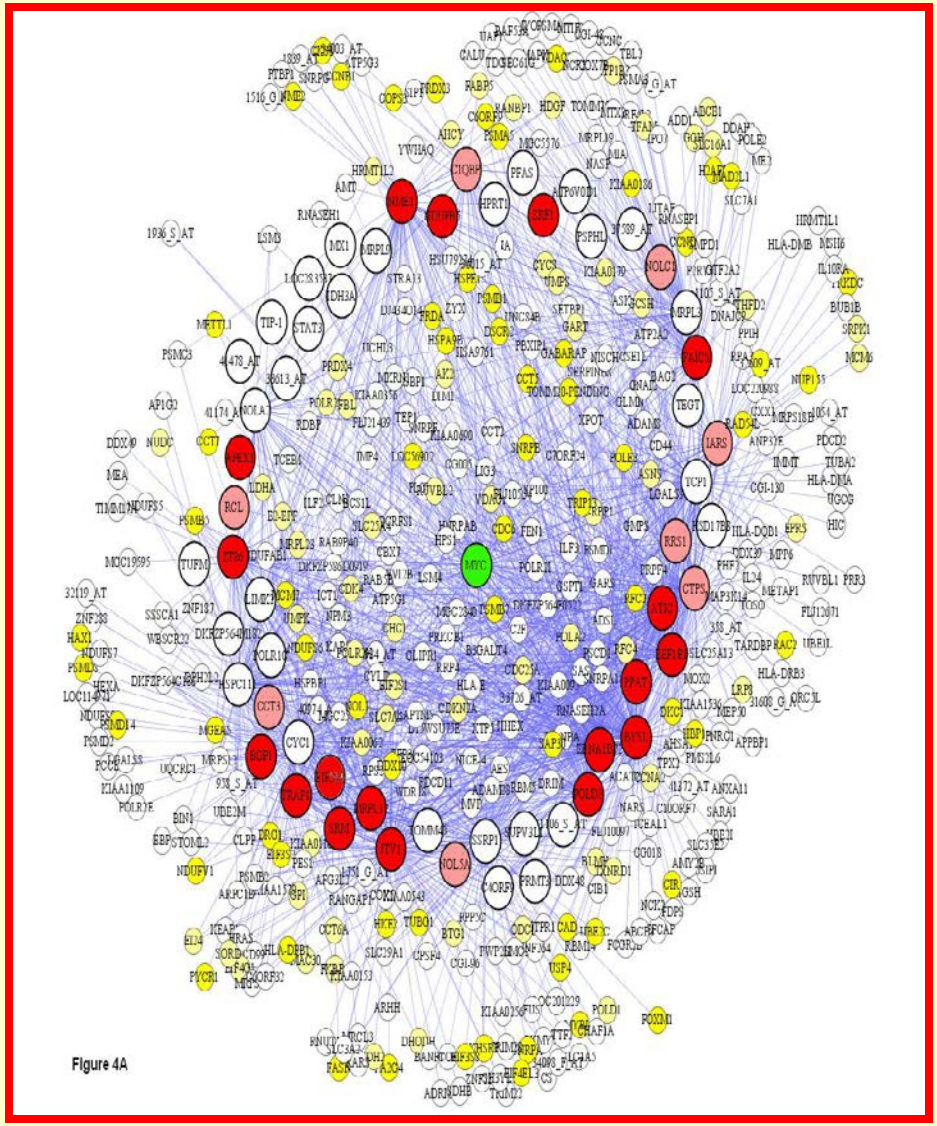
E. Mitochondrial Therapy (Pronuclear Transfer Approach).

F. Engineered Chromosomes.

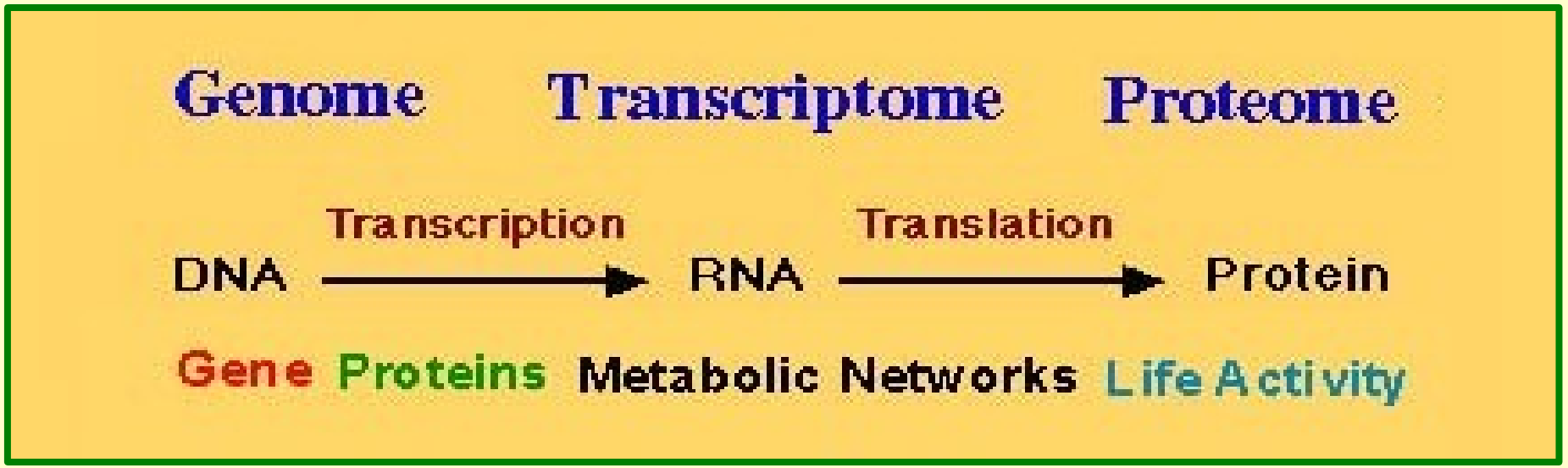
G. Protein Therapy (Molecular Chaperones).

The Concept Of Metabolic Networks





The Concept Of Genetic Therapy



Genetic therapy aims, primarily, at correcting the structural and the functional genetic defects that cause genetic diseases. Meanwhile, it also aims at offering genetically-based therapeutic approaches for non genetic diseases as well, like microbial infections and acquired organ failure.

In view of the extremely wide spectrum of pathogenetic mechanisms that underlie the development of genetic diseases, the list of therapeutic approaches to these disorders seems endless, at least theoretically. Currently, there are too many therapeutic approaches to genetic diseases either targeting the underlying pathogenetic mechanism(s) or aiming at reducing the side effects and deleterious consequences of these diseases secondary to altered pathophysiological states.

These therapeutic approaches include a wide variety of options including dietary management, drug therapy, replacement therapy, cell-tissue-organ transplantation, surgical intervention, and genetic therapy approaches.

As the term implies, genetic therapy aims, basically, at offering either radical cure or effective treatment of genetic diseases via correcting the underlying mutation-induced pathogenetic mechanisms of these diseases. Thus, this correction aims at targeting either the structural alterations of the genetic material at any of its organizational levels, or the resulting functional derangements.

Unfortunately, because of the extreme complexity of the structural organization(s) and the functional interactions of the human genetic material, quite few of these therapeutic techniques are worthy of trial. With the exception of the success of classic viral-based gene therapy for localized superficial melanoma of the skin, and the alleged success of stem cell therapy for a long list of genetic disorders, the way to safe and successful genetic therapy is still quite faraway from reality.

Based on our knowledge of these varied pathogenetic mechanisms, a wide variety of techniques have been theorized and tried at nearly all possible structural alterations and functional derangements levels : the gene level, the mRNA level, the protein synthesis level, as well as the master regulatory mechanisms that control all structural and functional aspects of the genetic material including the genome, both the nuclear and mitochondrial genomes, the transcriptome, and the proteome.

So, targets of genetic therapy include :

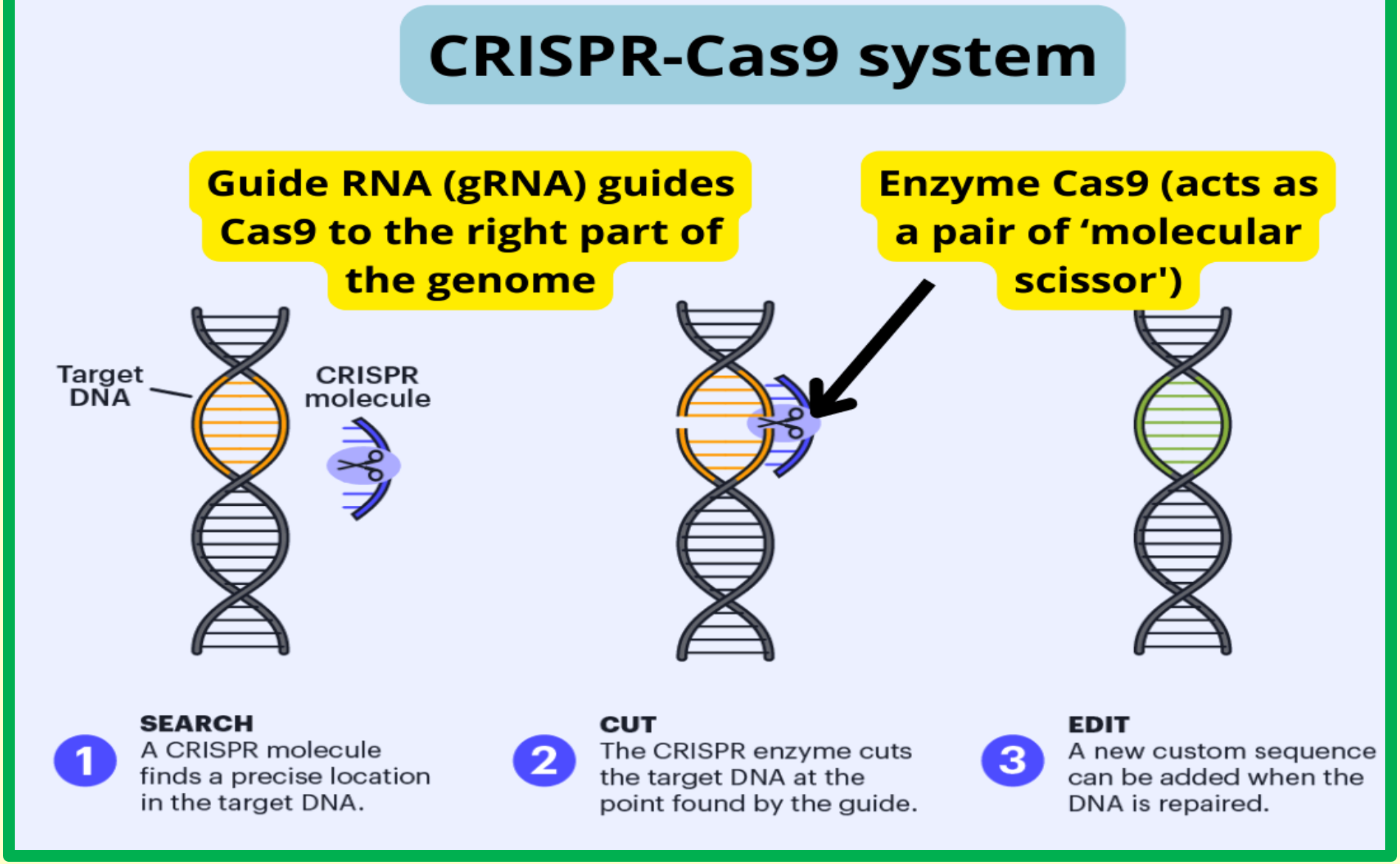
1. Single nuclear genes
2. Messenger RNA (mRNA)
3. Synthesized Proteins
4. Regulatory factors
5. Mitochondrial genome
6. The whole genome.

Methodological Approaches Of Genetic Therapy

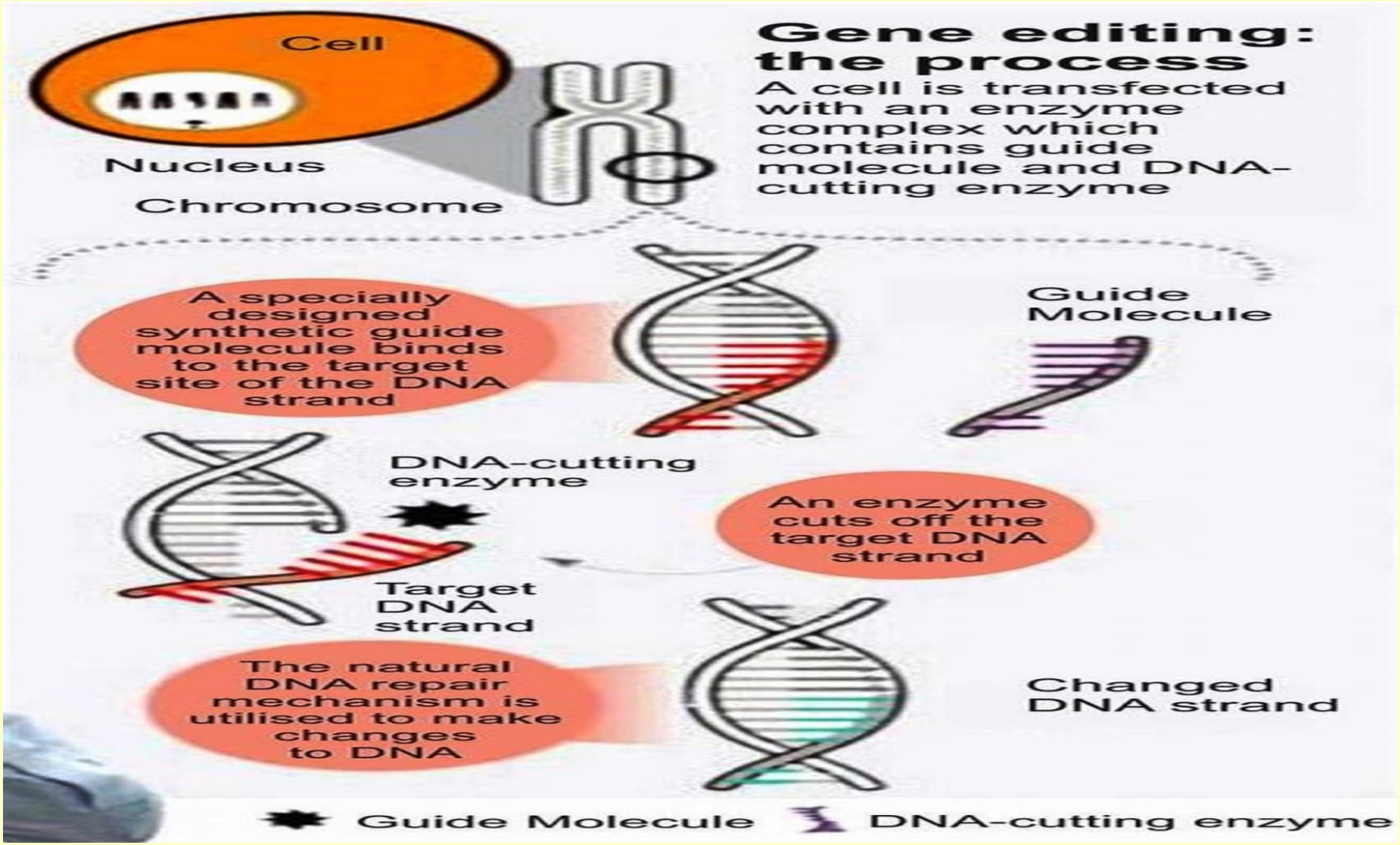
Many methodologies in genetic therapy techniques have been designed and tried depending on the nature, site and magnitude of the mutational event as well as on the nature, pathway(s) and consequences of the resulting pathogenetic mechanism(s). However, the main approaches to genetic therapy involved the following methodologies :

1. Gene Editing and Mutant Gene Repair techniques.
2. Transfer of normal genes to affected cells for single mutant gene disorders (single gene transfer approach).
3. Transfer of normal cells with normal genomes to patients with nearly any kind of genetic disease (whole genome transfer approach).
4. Pronuclear transfer in human embryos for treatment of mitochondrial diseases (mitochondrial genome replacement approach).
5. Messenger RNA (mRNA) repair-manipulation approaches.
6. Protein repair-manipulation approaches.
7. Methodologies tailored for specific therapeutic targets like induction of prophylactic mutations, drug potentiation, etc.

CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats) - Associated Enzyme 9.



Mechanism of Action of CRISPR Gene Editing Technique



Mechanism of Action of CRISPR Gene Editing Technique

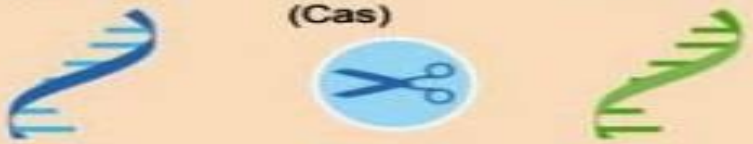
CRISPR-Cas can be thought of as a biological version of the 'search and replace' function in a word processor. It can be used to alter the DNA by cutting, replacing or adding pieces of genetic code.

An example of how the technique works

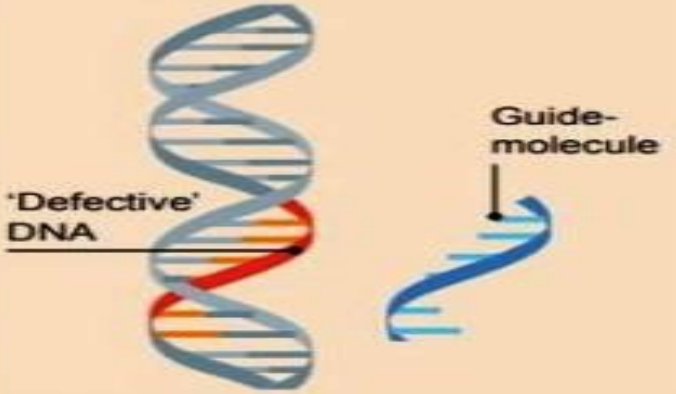
The genetic material in a cell (the DNA) contains an undesirable mutation.

The 'defective' DNA sequence can be changed with the help of a protein complex consisting of:

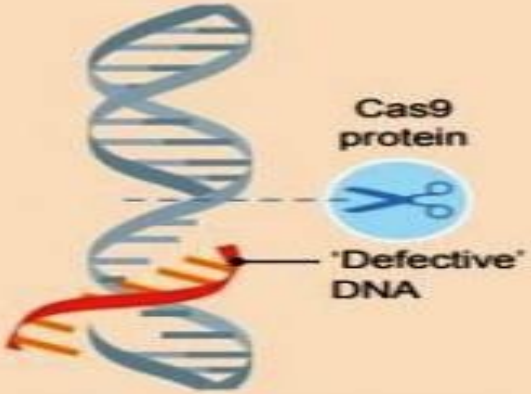
- a 'guide molecule' (CRISPR)
- a 'DNA-cutting' protein (Cas)
- a piece of DNA without the mutation



1. A specially designed guide molecule 'reads' the DNA in a cell until it finds a piece of 'defective' DNA that matches the sequence implanted in the guide molecule.

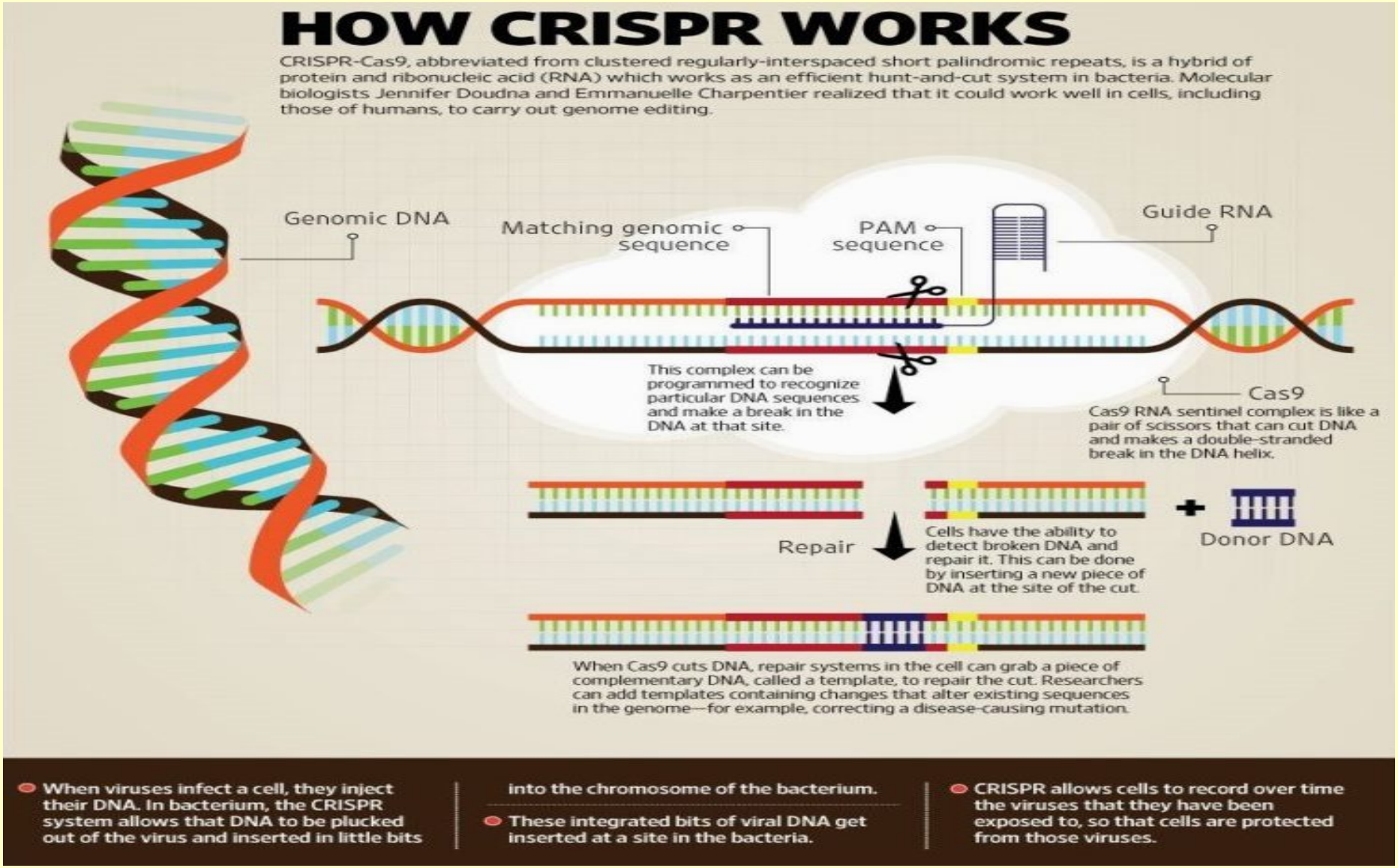


2. The Cas protein then cuts out this piece of DNA (deletion).



3. The deleted piece of DNA can now be replaced with a DNA sequence that does not have the undesirable mutation.

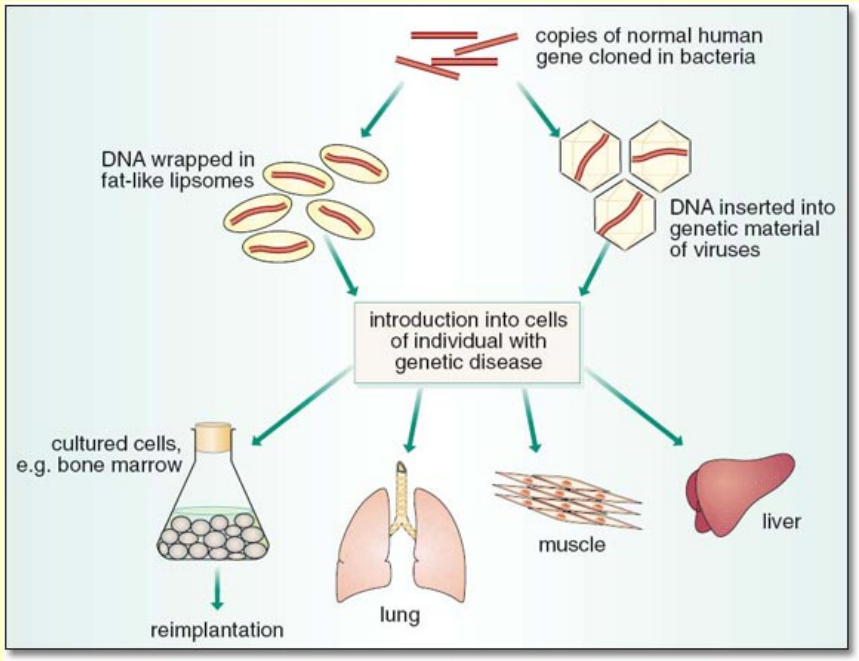
Mechanism of Action of CRISPR Gene Editing Technique



2. Single Gene Transfer Approaches

Theoretical Basis

The first trial of genetic therapy for single gene disorders was based on a simple, and apparently rational assumption : if a genetic disease is caused by a single nuclear gene mutation, then replacing the missing function(s) of the mutant gene, due to its deficient or defective protein product, with a normal gene extracted from normal cells and delivered or transferred to the nucleus of the cell, will correct the defect and cure the disease.



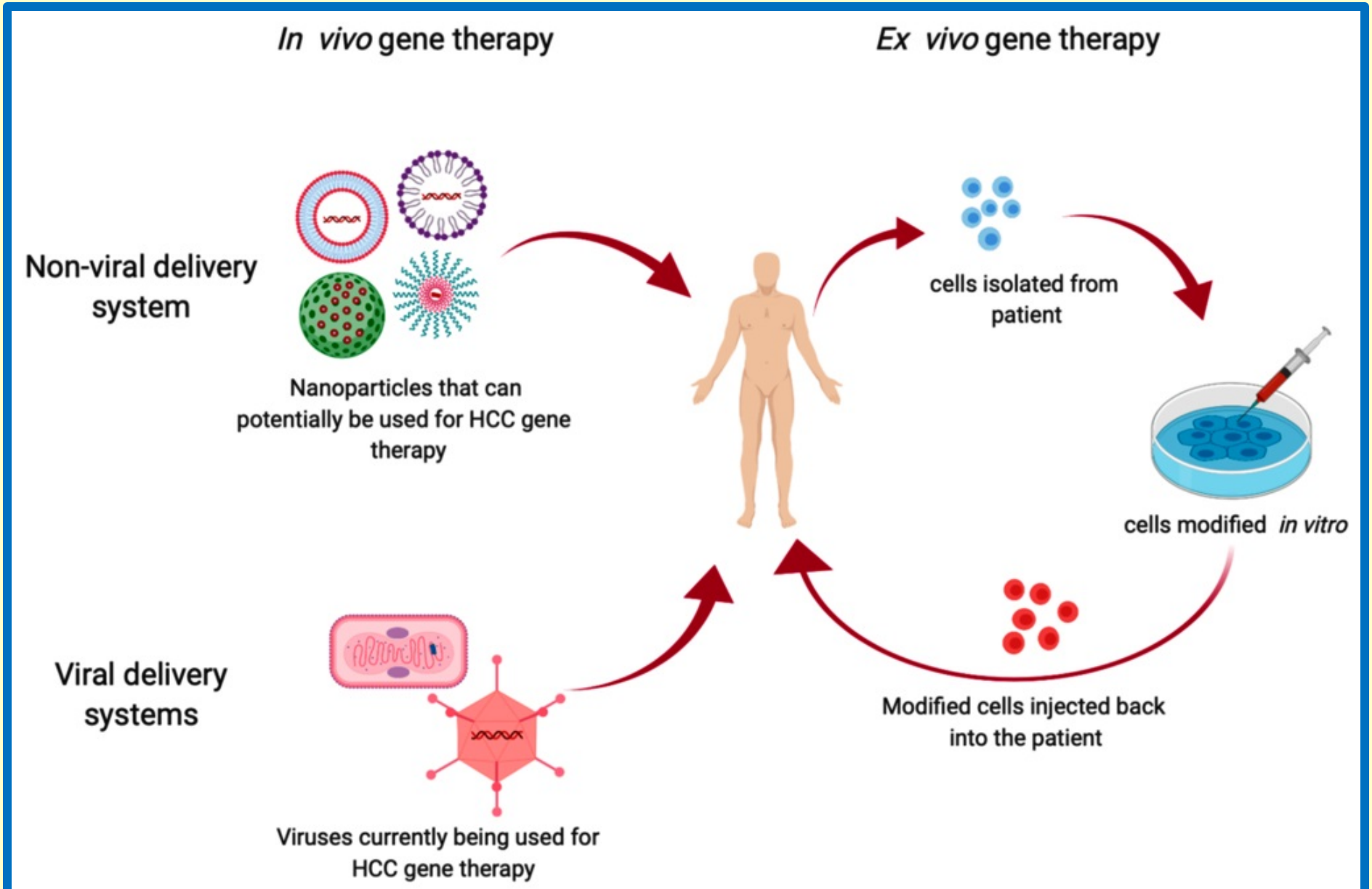
The rest of the assumption was based on the ability of the transferred or delivered gene, termed the **transgene**, to begin normal function in the host cell via the classic cascade of activation, transcription, translation and production of the deficient or defective gene product (protein, enzyme, regulatory factor) in proper configuration and sufficient amount to restore the functional or structural defects underlying the disease in question, thus offering a radical cure, or at least, effective treatment of the disease and amelioration of its side effects and complications.

Gene delivery systems

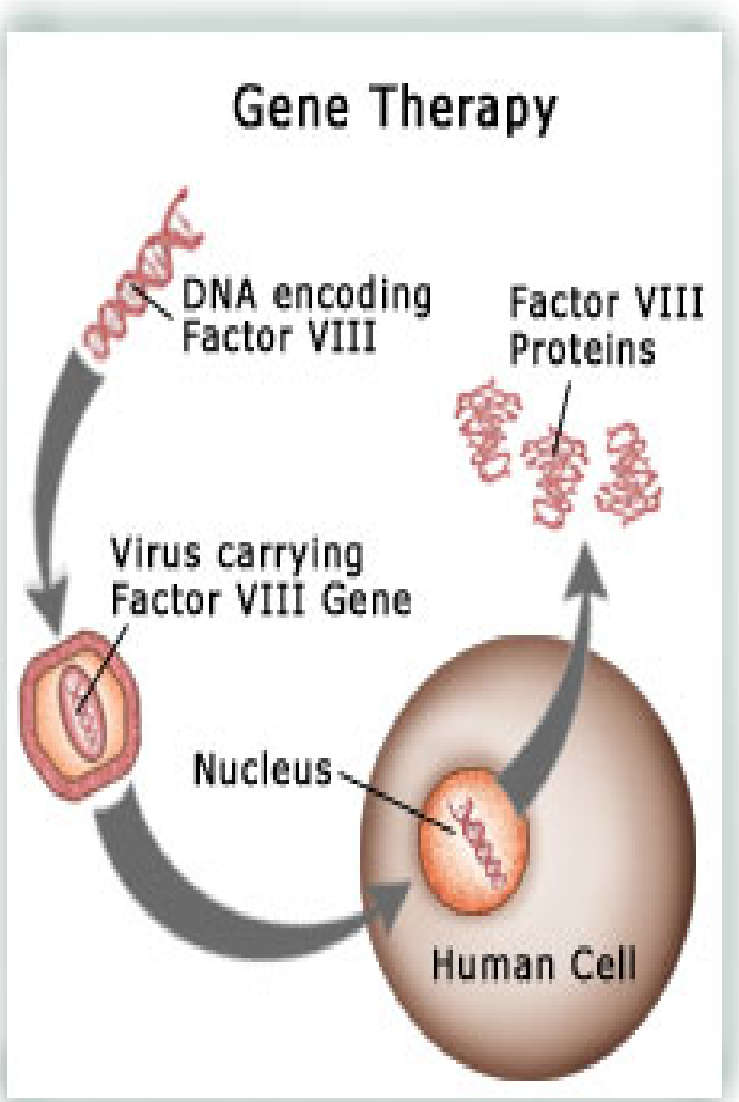
Four main gene delivery systems have been used in classic gene delivery techniques. These systems comprised :

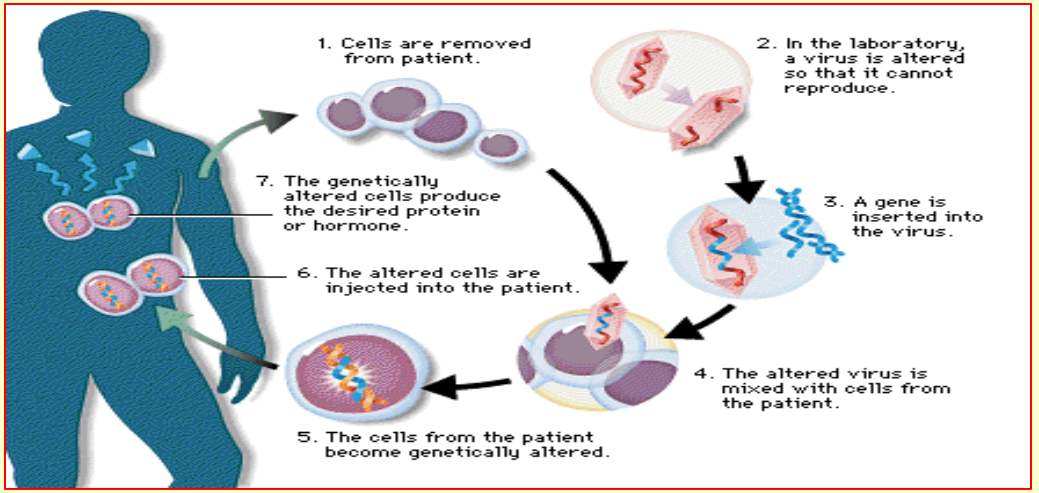
1. Viruses
2. Naked DNA (plasmids, cosmids).
3. Synthetic Liposomes
4. Synthetic molecular conjugates.

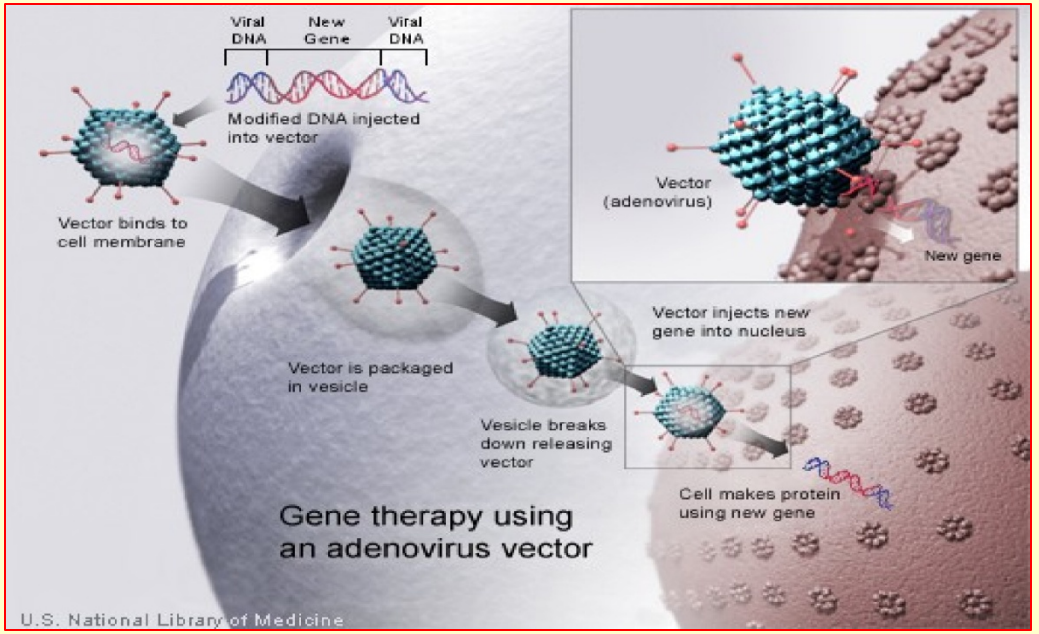
Techniques of Therapeutic Gene Delivery



Theoretical Bases Of Viral-Mediated Gene Therapy







1. Viral-mediated gene delivery

The introduction, or delivery, of a normal gene to the nuclear genome of the defective cell has been first tried using viruses as delivery vectors because they consist of nucleic acids, can infect cells and amalgamate their DNA with the host DNA (lysogenic phase) and can be modified or genetically engineered so that the required normal gene can be added to their genome and the viral genes responsible for pathogenic effects of the virus can be removed from its genome without affecting its ability to infect cells and deliver the normal gene to its genome.

The use of viruses in genetic therapy trials, however, revealed many disadvantages and serious side effects that compelled halting their use as gene delivery vectors. The most serious of these complications is the widespread insertional mutagenesis due to their haphazard delivery to the nuclear genome of target cells. The induced mutagenic events results in widespread damage to the genome leading to disturbed gene functions with consequent pathophysiological alterations in cell functions depending on the nature and magnitude of the genomic damage.

Malignant transformations of infected cells due to inactivation of tumor suppressor genes, activation of proto-oncogenes or damage of genes responsible for regulating the cell cycle were among the common complications of viral-mediated gene therapy trials that put a clear-cut end to their use in this therapeutic approach. Other side effects included immunodeficiency, organ failure and toxicity, among many others.

2. Non-viral mediated gene delivery

Other non-viral gene delivery vectors have been used in single gene therapy trials. They included : naked DNA genes in the form of plasmids or cosmids, liposomes and molecular conjugates. Compared to viruses, these vectors are relatively safer, non-infectious, non or less mutagenic, less damaging to the genome of infected cells, and do not elicit immunological reactions in their host. They have a much larger capacity than viruses enabling delivery of large genes and their large scale production is possible using pharmaceutical techniques. However, because they do not integrate into the host genome they have a short duration of effects limited by the life cycle of the host cell.

PROSPECTS OF SINGLE GENE TRANSFER METHODOLOGY

Because of the many serious complications, side effects and disadvantages of this technique of gene delivery, it is not used any more in this regard. In fact, its theoretical design for human gene therapy was a fatal ethical mistake and an irresponsible evidence-lacking proposal from the start in view of the extreme complexity of the structural organization of the human genome, the more complex organization of the huge number of genetically-controlled metabolic-regulatory networks mediating cellular functions, the haphazard manipulation of such a complicated dynamic system and, last but not least, our still markedly deficient knowledge of most structural and functional aspects of the human genome.

The failure of this approach in achieving any success after three decades of its invention put an end to its use in gene therapy trials except for experimental ex-vivo trials in animals and lower forms of life within the promising context of genetic engineering and pharmaceutical applications.

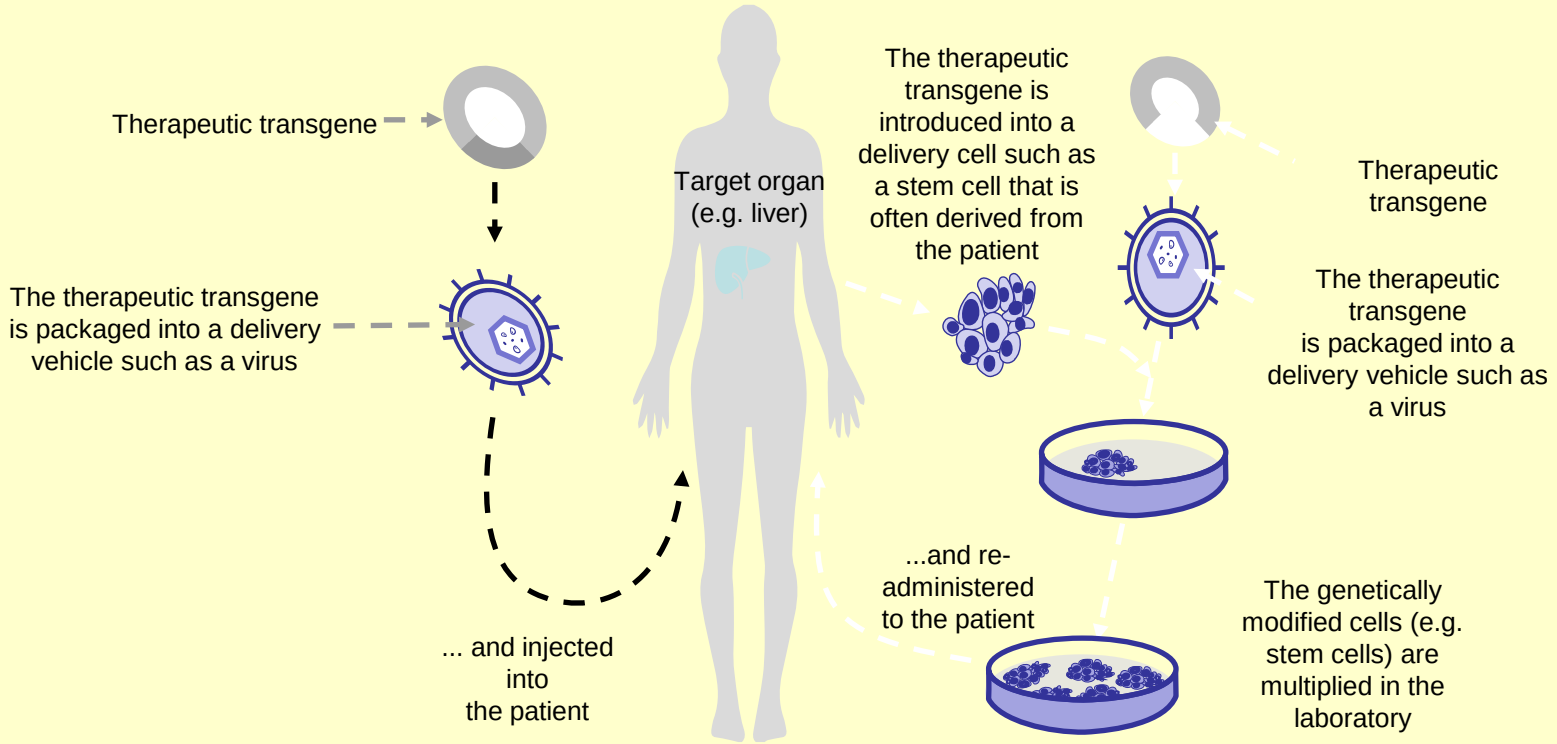
Gene Therapy Delivery

Direct Delivery

Cells are extracted from the patient, modified with the
therapeutic gene, and injected back into the patient

Cell-Based

The therapeutic gene is transferred directly to target
cells in the body

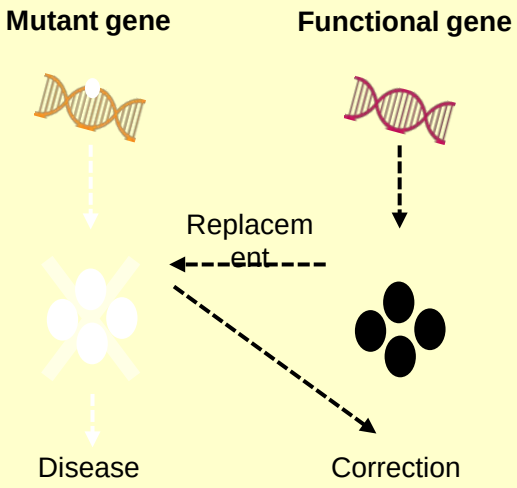


Collins M & Thrasher A. Proc Biol Sci 2015;282(1821).

Approaches To Gene Therapy (1)

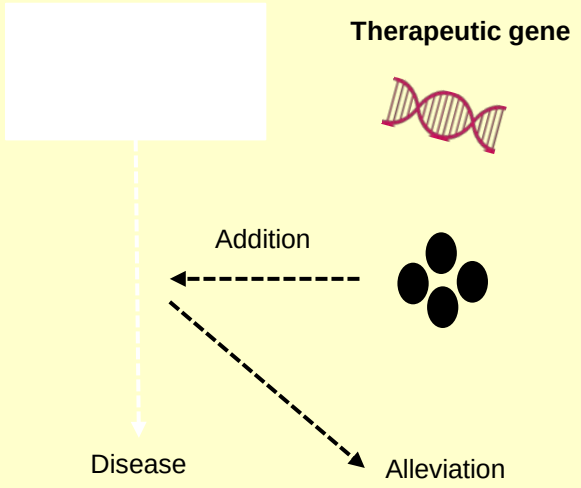
Gene Replacement Therapy

For monogenic diseases; involves replacing a mutated gene that causes disease with a healthy gene



Gene Addition

For complex and infectious diseases; introduces a new gene into the body to help fight a disease, often to supplement a targeted therapeutic agent

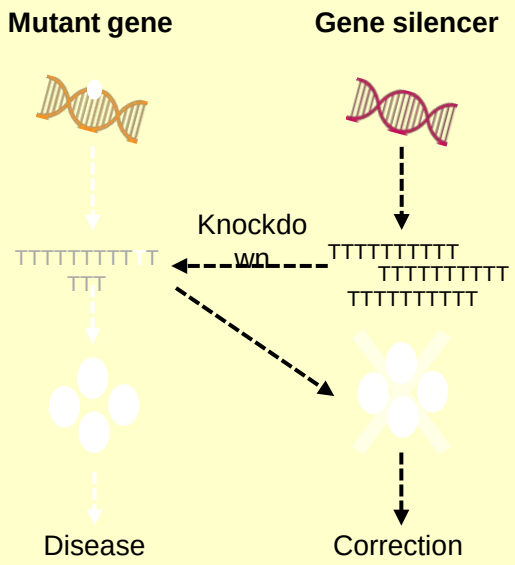


Reproduced from Wang D, et al. Discov Med 2014;18:151–161.

Approaches To Gene Therapy (2)

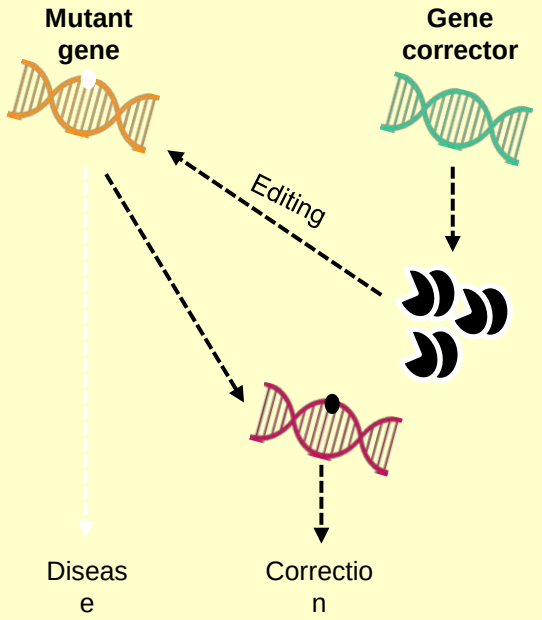
Gene Inhibition or “Knockdown”

Inactivating a mutated gene that is over-producing its product by targeting RNA



Gene Editing

Making a targeted change to the gene sequence



Reproduced from Wang D, et al. *Discov Med* 2014;18:151–161.

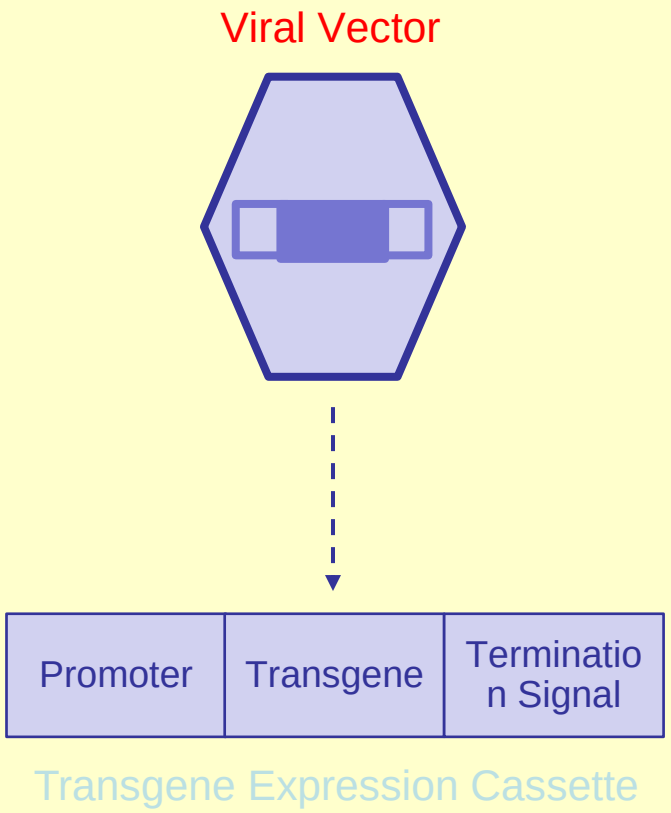
GRT Components (*In Vivo* Approach)

Three key components:

- the **vector**, or vehicle, which is injected into the patient and by which a transgene is delivered to the targeted cells[1]
- the **transgene**, which is a sequence of complementary DNA (cDNA) coding the replacement gene[1]
- the **promoter**, which is the DNA sequence
  that acts as a “turn on” switch and modulates
  the expression of the transgene[1]

Also typically includes, either:

- A **termination signal** to end gene transcription[1], or
- **Inverted terminal repeats (ITRs)** at either end of
  the cassette to allow for synthesis of complementary DNA[2]



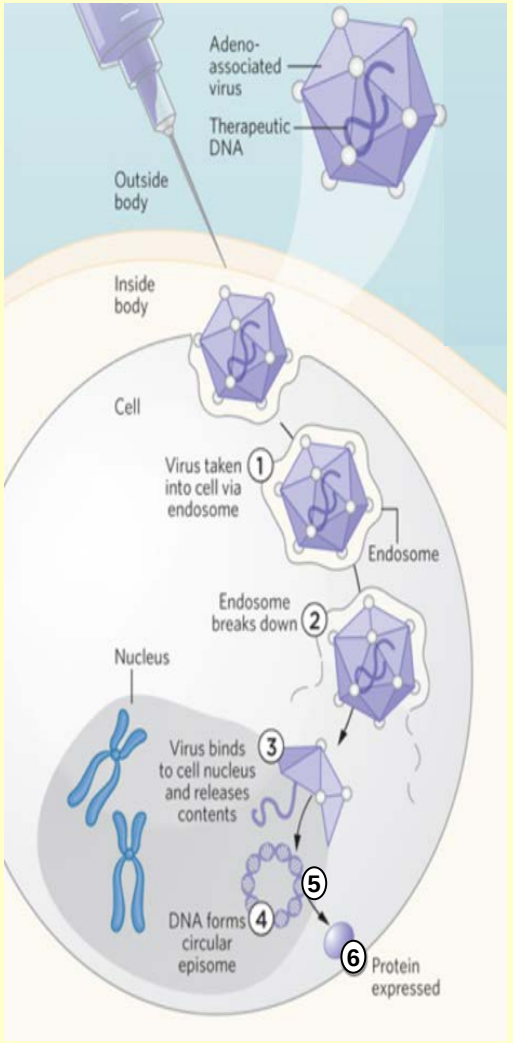
Adapted from Wang D. *Discov Med* 2014;18:67–77.

GRT, gene replacement therapy.
1. Wang D, et al. Discov Med 2014;18:67–77.
2. Ayuso E, et al. Curr Gene Ther 2010;10:423–436.

AAV Gene Therapy

AAVs deliver genes without integrating them into the genome

1. Virus is taken into the cell via the endosome
2. The endosome breaks down
3. Therapeutic DNA enters cell nucleus as a double-stranded molecule ready for transcription
4. Therapeutic DNA forms a circular episome
5. Upon promoter activation, transcription occurs
6. The resulting transcript leaves the nucleus and travels to the ribosome for translation (protein synthesis)



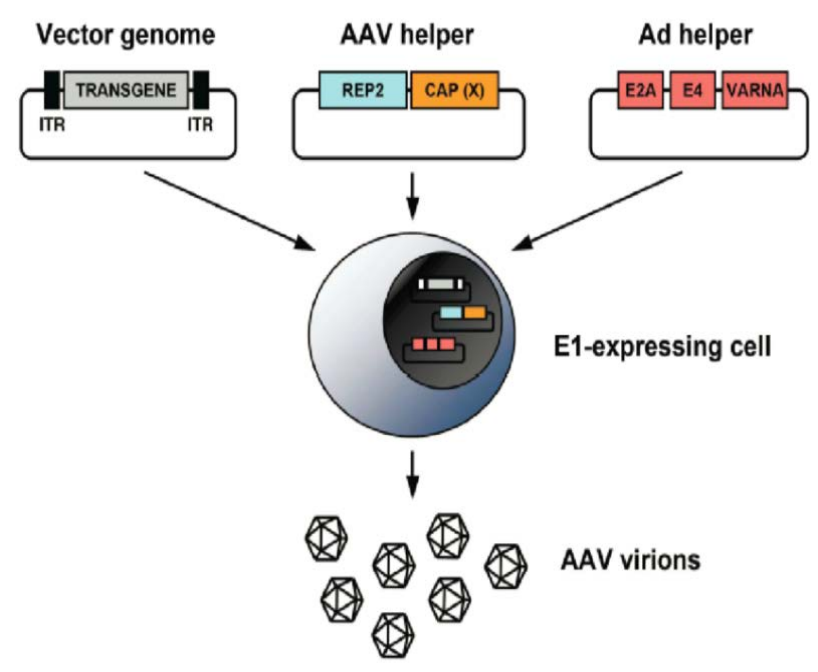
Graphic from Akst J. *The Scientist*. June 2012.
Naso MF, et al. BioDrugs 2017;31:317–334.

AAV Production Overview: Development & Production

The AAV production process is complex and sophisticated

1. Transgene Development[1] --------→ 2. AAV GRT Production[2] --------→ 3. Concentration & Purification[2]
4. Quality Assurance[3,4]

cDNA is reverse transcribed from human mRNA (no introns)



Purification produces vectors with high titer, high potency, and high purity[2]

QA using FDA-established requirements and predetermined specifications[3,4]

Graphic from Ayuso E, et al. *Curr Gene Ther* 2010;10:423–436.

1. McArty DM, et al. Mol Ther 2008;16:1648–1656.
2. Ayuso E, et al. Curr Gene Ther 2010;10:423–436.

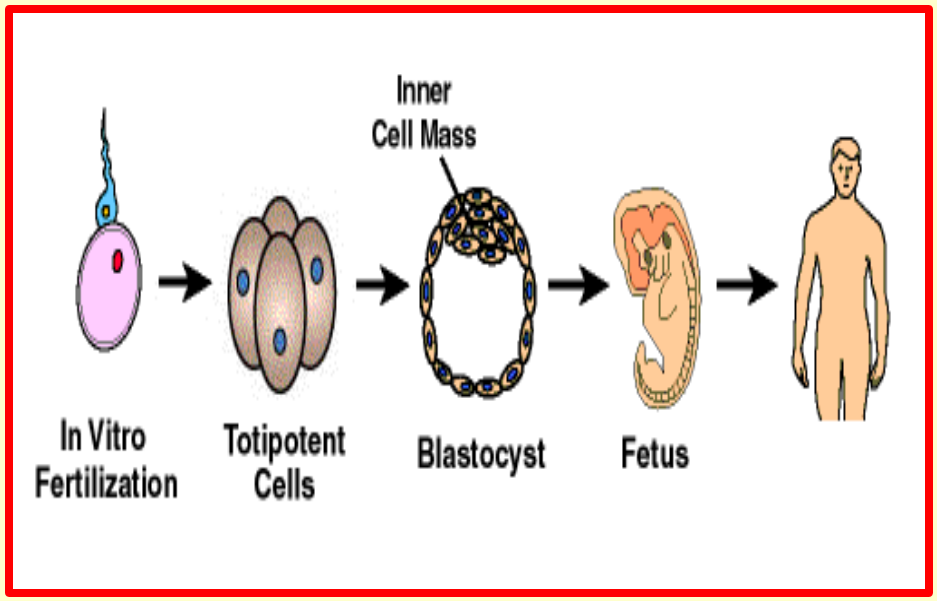
3. Whole Genome Transfer Approaches

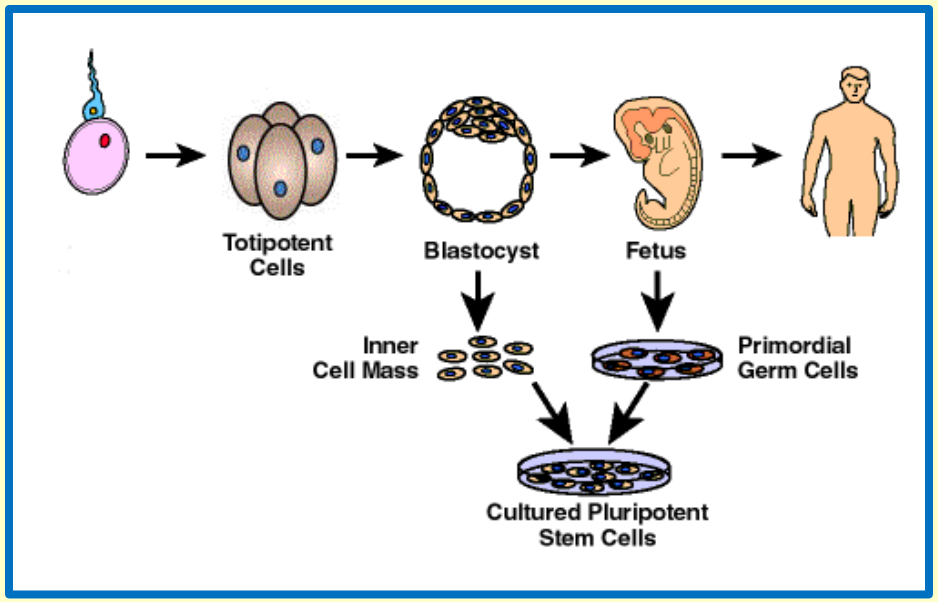
Theoretical Basis

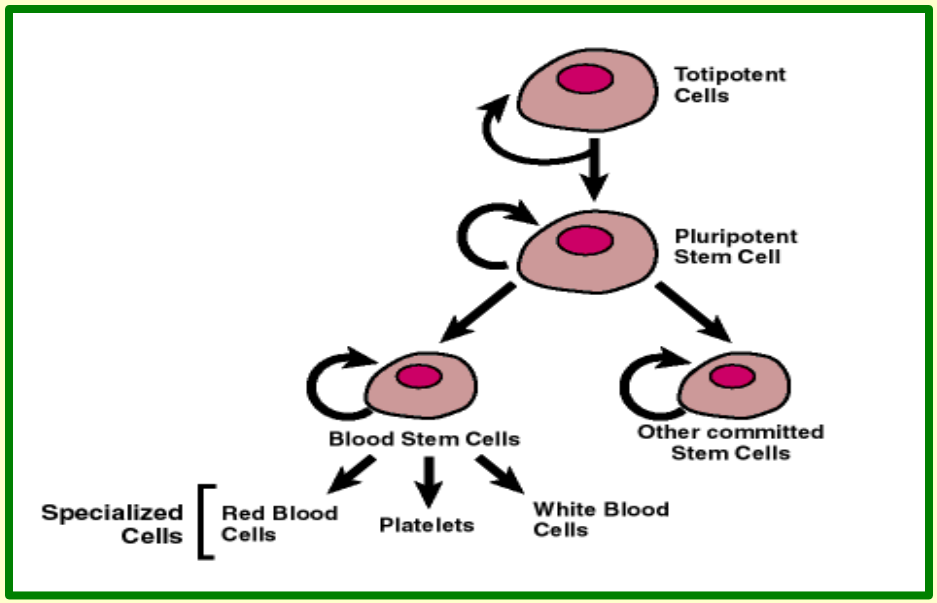
2. Transfer of normal cells with normal genomes to patients with nearly any kind of genetic disease (whole genome transfer approach) has emerged as a more rational, safer, more reliable and a global approach in genetic therapy after the unsuccessful outcome of single gene transfer approaches. This methodology in its crude design has been, and is still being, tried successfully since decades as a cell-tissue-organ replacement therapy as bone marrow, kidney and liver transplantation and is progressively extending to include many other tissues and organs.

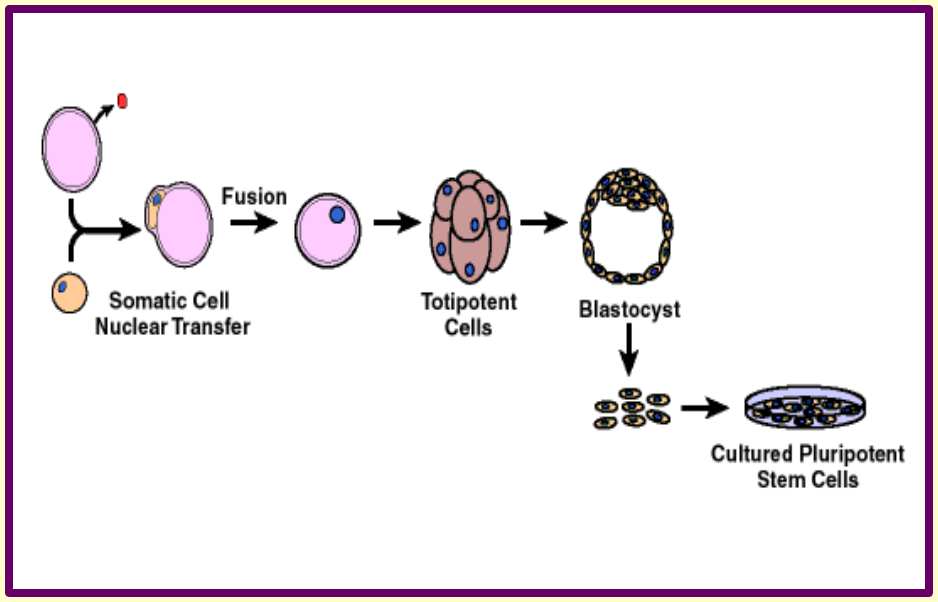
In essence, stem cell therapy approach represents the fine tuning of this therapeutic modality. However, the limitations of this methodology are conspicuous and confine its current therapeutic potential to their use as a source of normal cells with fully functional genomes that can differentiate to more specialized cell types to replace damaged cells, tissues or organs.

Stem Cells : Basic Considerations









The limitations of stem cell therapy approach are, mostly, due to lack of reliable techniques that guarantee precise control over their behavior as new isolated genetic systems after their transfer, even to their source of origin.

The infinite capability of stem cells to differentiate to any cell kind is a great merit in therapy, only if this differentiation is selective and directed within the context of a specific multifactorial environment along specific complicated morphogenetic pathways. This environment is properly availed only during the intrauterine phase of fetal development and can never be mimicked again under different conditions.

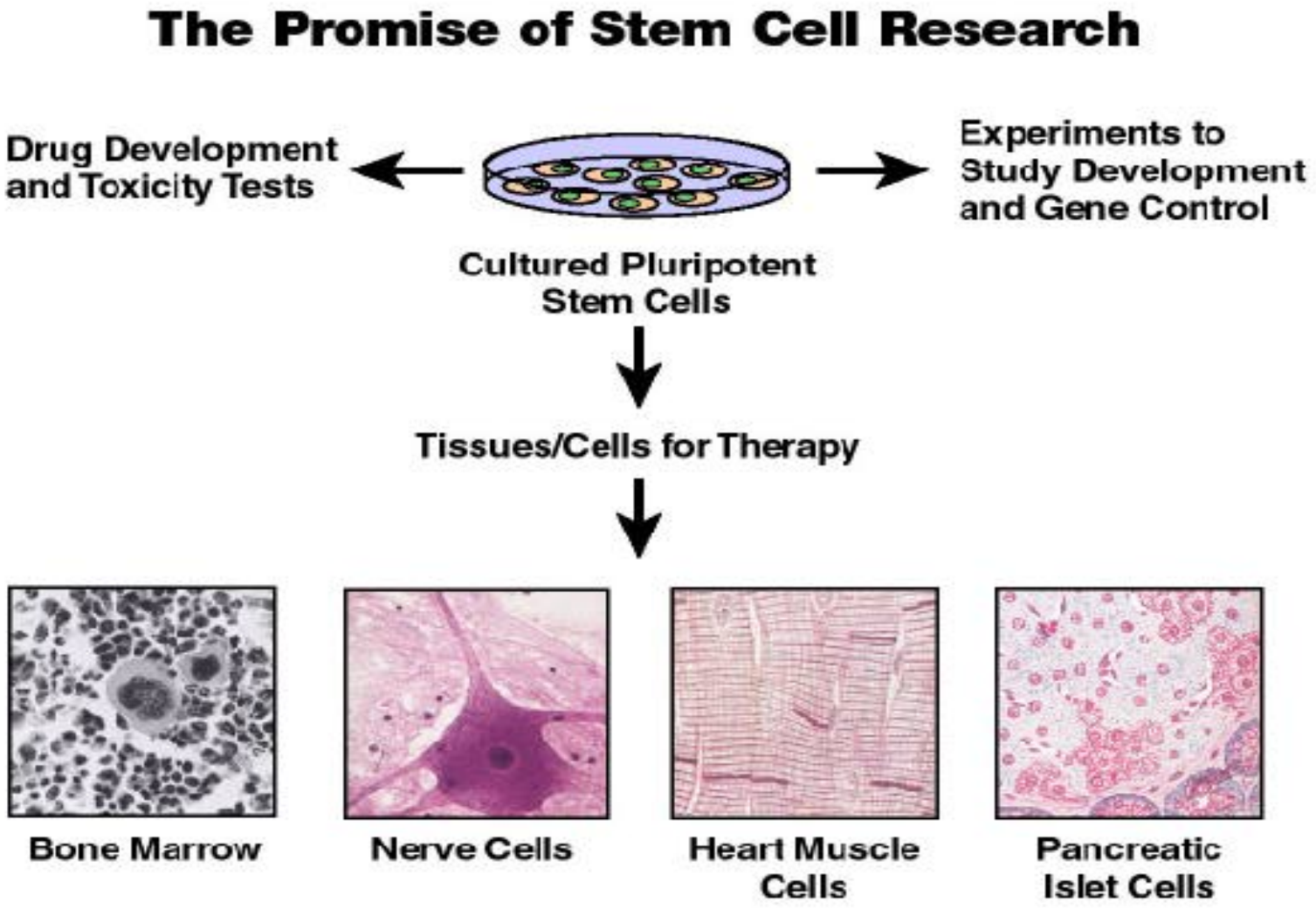
Thousands of genes participate in development of single embryonic and fetal organs under strict regulation exerted by a very large number of master genes, whose structures, locations and modes of action of most of them are, still, unknown. Accordingly, the possibility of transferred stem cells to form complete tissues or organs seems unrealistic and is an evidence-lacking assumption.

The Promise Of Stem Cells In Genetic Therapy

The hopeful promise in stem cell research rests on the potential application of stem cells in making cells and tissues for medical therapies. Today, donated organs and tissues are often used to replace those that are diseased or destroyed. Unfortunately, the number of people needing a transplant far exceeds the number of organs available for transplantation. Pluripotent stem cells offer the possibility of a renewable source of replacement cells and tissues to treat a myriad of genetically-determined and non-genetic diseases, conditions, and disabilities including amyotrophic lateral sclerosis, Parkinson's disease, spinal cord injury, burns, heart disease, diabetes mellitus, arthritis and many others.

Stem cell research has a great promise in prophylactic genetics as it will help us understand how they transform into specialized cells that make a human being. Some of the most serious conditions, such as cancer and birth defects, are due to defects that occur during this process. A better understanding of normal cell development will allow us to understand and perhaps correct the errors that cause these diseases.

Stem Cells Therapeutic Approaches



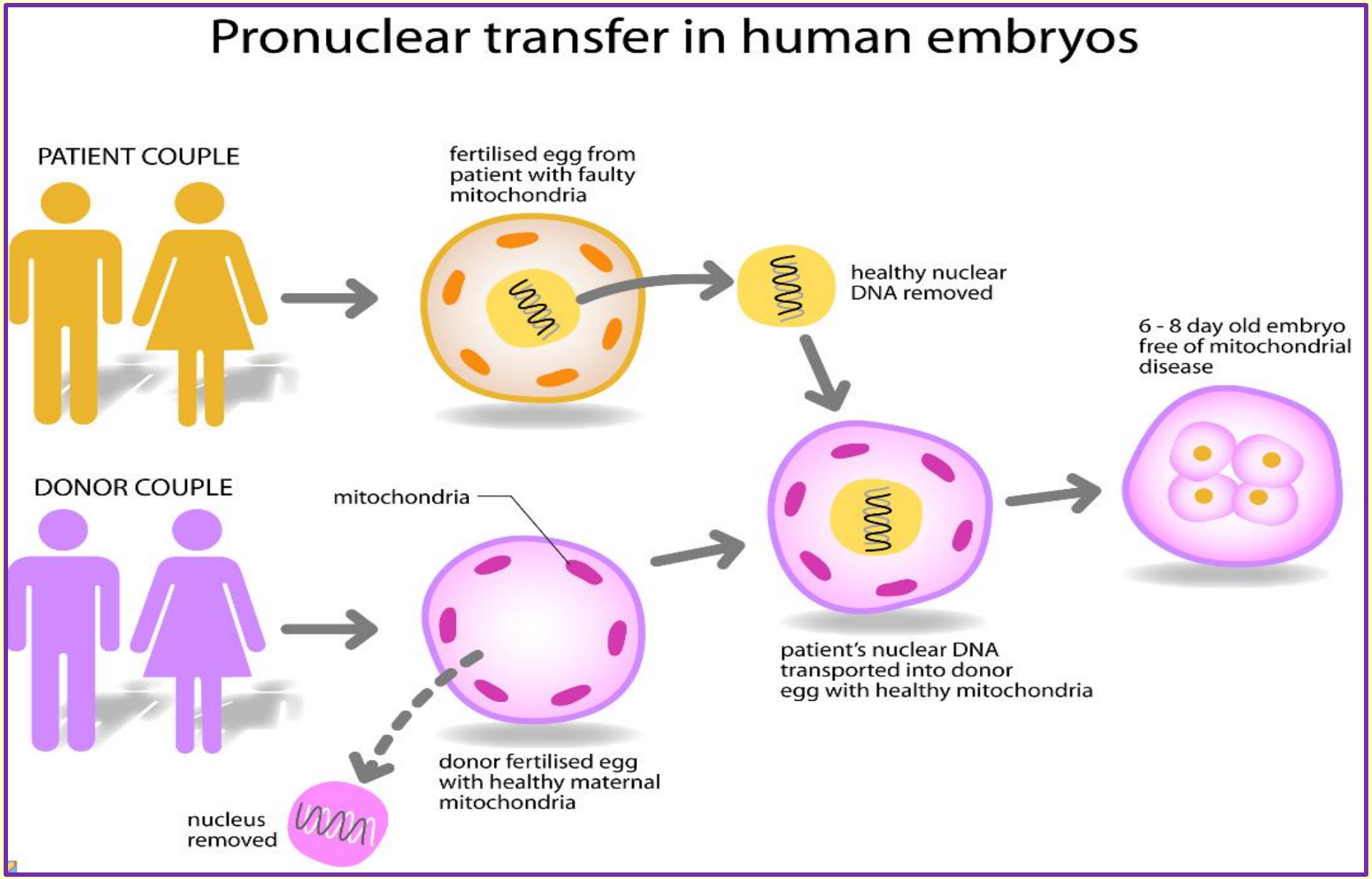
4. Mitochondrial Genome Replacement Approach

Theoretical Basis

The discovery that mitochondrial diseases result from mutations in mitochondrial genes evoked many therapeutic trials. The assumption that replacing defective mitochondria with normal ones via the technique of pronuclear transfer of normal nuclei with normal genomes in human embryos for treatment of mitochondrial diseases proved to be an effective therapeutic modality whereby the nucleus of a zygote of a couple with a maternal mitochondrial disorder is extracted and transferred to a zygote of a normal donor couple after removing its nucleus. The donor zygote, containing the free nuclear genome of the original couple and the free mitochondrial genome of the donor couple is implanted in the uterus of the original mother where it develop into a normal offspring free of the mitochondrial disease.

This genetic therapy technique is curative for mitochondrial diseases due to mutations only affecting mtDNA. It is not applicable, for instance, for oxidative phosphorylation defects due to nuclear gene mutations or combined nuclear and mitochondrial gene mutations.

Pronuclear Transfer Approach For Mitochondrial Disorders



Risks And Limitations Of Pronuclear Transfer Approach

In spite of its simple and rational bases, potential risks of pronuclear transfer methodology in genetic therapy of mitochondrial diseases are considerable, because there is no guarantee that the new mitochondrial genome is free of any hidden or undetected mutations. This is due to the peculiar clinical threshold feature of mitochondrial diseases as well as the characteristic heteroplasmy ststus of mtDNA which makes any trial for mutation screening of mtDNA a practical impossibility. This risk could be a real threat of affection with a mitochondrial disease to the developing embryo if the new mitochondrial genome has a mutation whilst it is being incorporated in the new zygote during the bottle neck phase of fertilization.

Further, albeit subtle, disadvantages of this approach are due the inconvenience it imposes because of the necessity of performing IVF trials for both the donor and recipient families, and also due to the high financial costs of the procedure, making it a highly selective and effective option, though a potentially risky approach towards genetic therapy of mitochondrial diseases.

5. Messenger RNA (mRNA) Repair-Manipulation Approaches

Theoretical Basis

Current methodologies focus their impact on mRNA as a final target in genetic therapy approaches. The reasons for this strategy are obvious in view of the peculiar characteristics of mRNA and the advantages of the techniques over other treatment trials.

First, manipulation of mRNA happens away from the nucleus, thus obviating the need for targeting nuclear genes via classic gene transfer techniques, and nullifies the potential risks of insertional mutagenesis, disturbed genomic stability and the serious consequent disarray of cellular functions which might proceed to malignant transformation or even to cell death.

Second, mRNA is abundant in the cytoplasm and can be extracted with ease in pure form and in large amounts as well. This allows for its characterization, sequencing, detection of mutations and preparation of complementary molecules devoid of the mutations in large amounts sufficient for therapeutic applications.

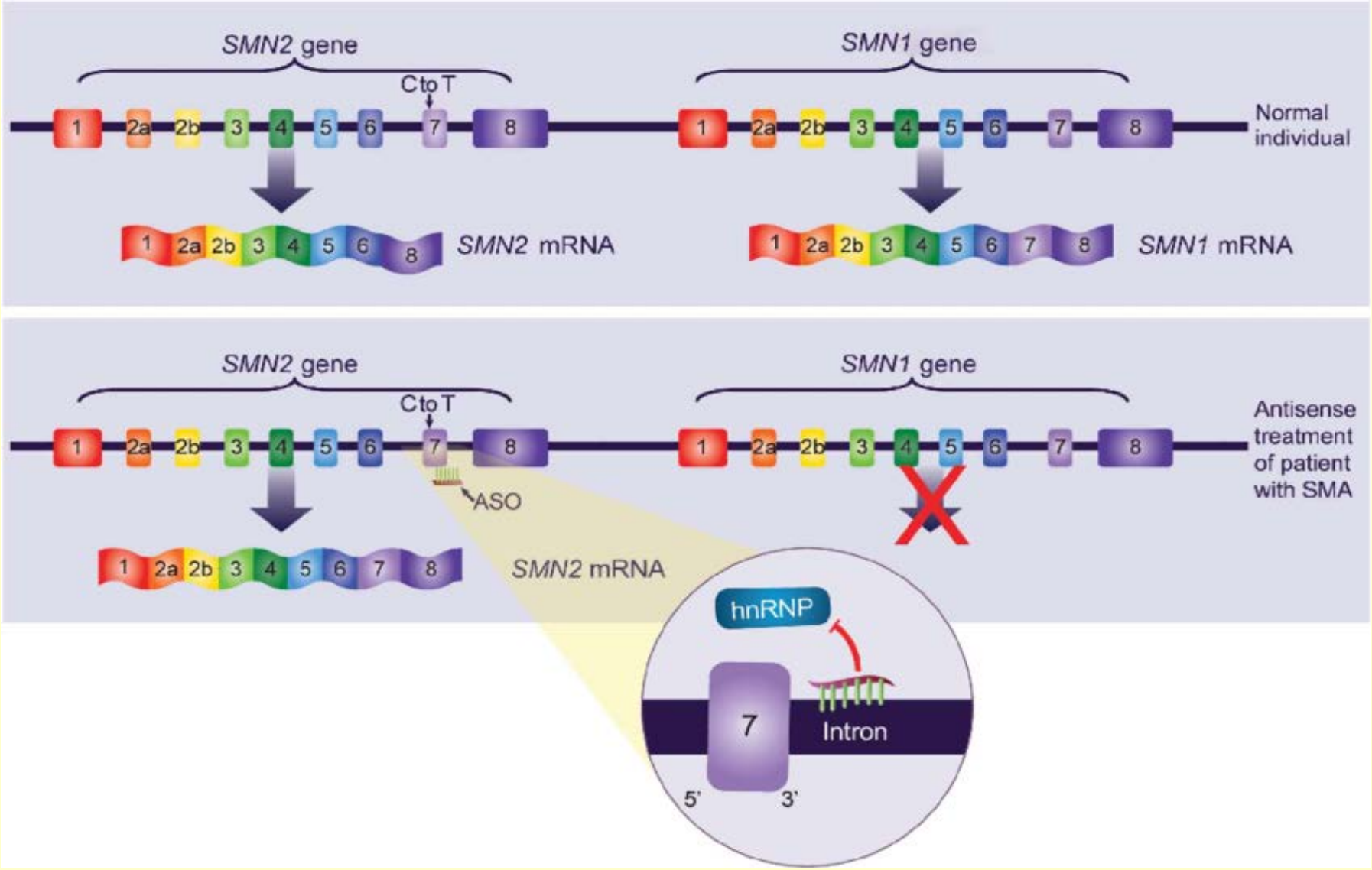
Third, mRNA acts as the final source of genetic information needed for protein synthesis. Transcription of defective mRNA by mutant genes represents the commonest pathogenetic mechanism that underlie the development of most genetic diseases, and mRNA repair by microRNA molecules, like guide RNA, can be achieved much more easily than trying to repair the a mutant nuclear gene.

Overproduction of proteins that mediate the malignant behavior of cancer cells (oncoproteins) and disturb all normal cellular metabolic-regulatory networks constitute the core of the malignant phenotype. This overproduction occurs due to over-expression of the oncogenes, hyper-transcription of mRNA and overproduction of the corresponding oncoproteins. Accordingly, stopping the translation of the excess mRNA in cancer cells, leading to consequent arrest of overproduction of oncoproteins, represents a pivotal approach towards radical treatment of tumors. Currently, most valid genetic therapy trials of cancer are technically designed to achieve this goal via a multitude of hypothesis e.g. use of mRNA antisense oligonucleotides, use of mRNA-specific exo-nucleases, use of poly-Thymine constructs to join with the poly-Adenine tail of the mRNA leading to its enhanced degradation, etc.

The number of experimental genetic therapy approaches and actual trials involving manipulation of mRNA as their final target comprises an endless and ever expanding list of techniques. Literally, tens of such trials have been hypothesized and are under experimental validation, but only few of them are evidence-based and seem to be promising in this regard. These approaches include :

1. Correction of mRNA defects by repair-specific micro or small RNAs.
2. The use of hammerhead ribozyme to induce site-specific cleavage of mRNA to down-regulate the expression of mutant alleles.
3. The use of antisense oligonucleotides to interfere with mRNA decoding and translation.
4. The use of single or multiple exon skipping approach in some specific disease conditions (e.g. Duchenne myopathy) to allow for synthesis of a less defective, relatively more efficient functional protein.
5. The use of mRNA stabilizer factors.
6. The use of mRNA destabilizing factors.
7. The use of signal transducer molecules to enhance and/or silence translation, attachment to rRNA or tRNA.

Mechanism of Action of Nusinersen



6. Protein Repair-Manipulation Approaches

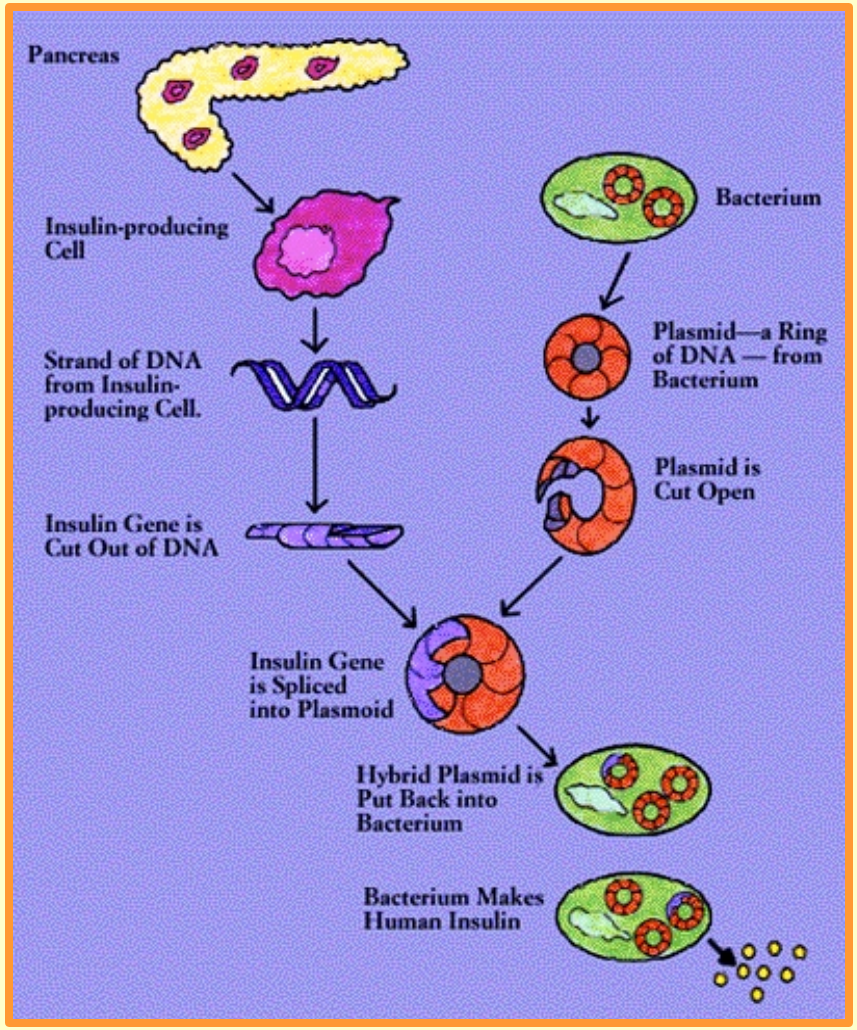
Genetic disorders are caused by mutations of the genetic material but their pathogenesis is mediated exclusively by the pathophysiological alterations of the metabolic-regulatory networks that regulate all aspects of cellular functions. These alterations represent the ultimate consequences of deficient protein synthesis or synthesis of defective proteins due to the mutations.

Genetic therapy approaches targeting protein synthesis (translation) defects aim at either correction of structural defects of translated proteins or at increasing the rate of production of deficient proteins. The first target is a molecular mimicry of the protein repair mechanisms in the cell whereby defective proteins could be corrected, within limits, by the chaperons which are specific protein moieties capable of correcting some structural protein defects, e.g. misfolding defects. Defective proteins that can not be corrected by the chaperons are dealt with via other pathways, most important of which is the ubiquitination-proteasome degradation pathway where they are degraded by specific proteases to smaller peptides and finally to amino acids that can be reused again in cellular metabolism.

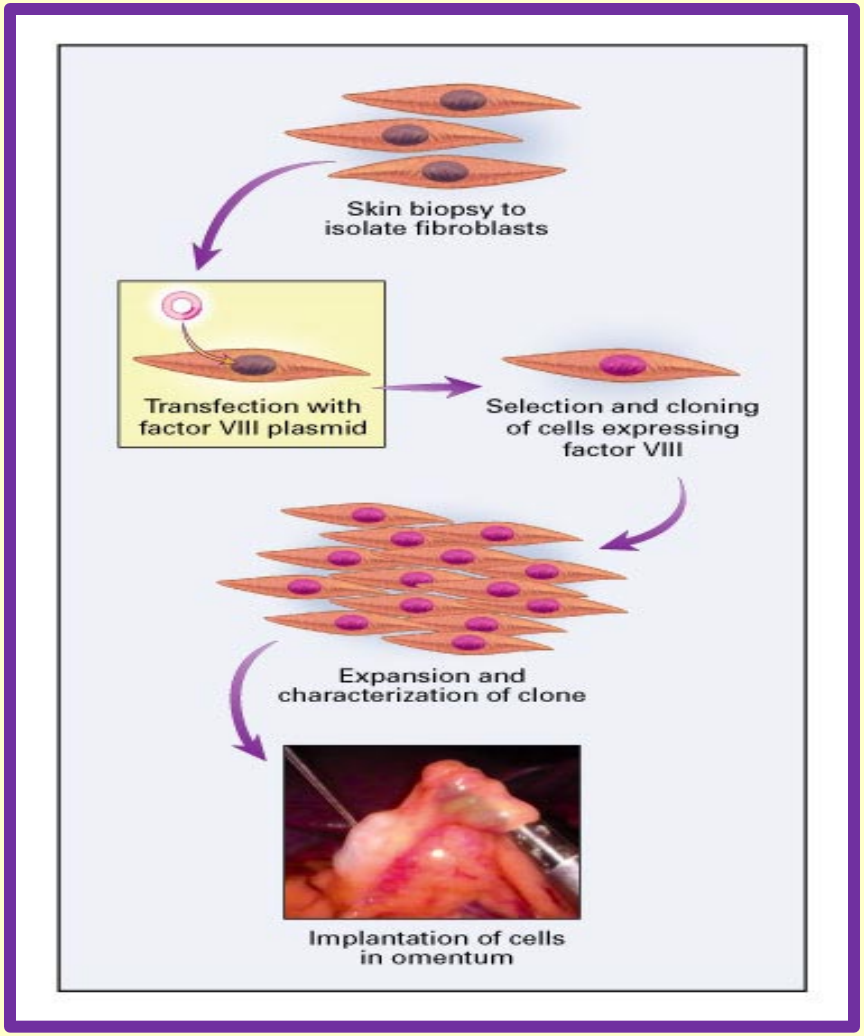
In view of this scheme revealing the role of the proteome in cellular activities many experimental approaches have been tried to offer genetic therapy for diseases due to protein disorders. In fact, the direct provision of intact proteins to patients with these diseases was the first treatment approach practiced since decades long before the emergence of the concept of genetic therapy of these diseases, and is still the most reliable and most effective and safe treatment modality of these patients. Prominent examples of this approach include the use of anti-hemophilic globulin, immunoglobulins, insulin, growth hormone, and many other therapeutic proteins in corresponding genetic disorders due to their deficient production.

In spite of this success, the need for genetic therapy for these diseases stemmed in view of the advantages such a therapy can offer to these patients, notably obviating the everlasting persistent need for repeated lifelong administration and avoiding the potential risks associated with development of harmful immunological reactions and the possibility of progressive reduction in their therapeutic efficacy over long time administration. Actually, the need for this therapeutic approach is crucial in view of the unavailability of such therapeutic proteins for the majority of genetically-determined protein deficiency disorders.

Therapeutic Protein Production By Genetic Engineering



Genetic Therapy Approach Of Factor VIII Deficiency



Many genetic therapy approaches in this respect have been tried including :

1. The use of pharmaceutical molecular chaperons to correct post-translational modifications defects which underlie the pathogenesis of a considerable number of common and serious genetic diseases like Alzheimer's disease, Parkinson's disease, Creutzfeldt–Jakob disease, Huntington's disease, cystic fibrosis, Gaucher's disease and many other degenerative and neurodegenerative disorders.

2. Manipulation of the translation machine in the cytosol by signal transducers to ensure proper configuration of synthesized proteins.

3. Construction of mRNA stabilizing factors that prolong the life span of mRNA allowing for more repeated cycles of translation and severalfold increase in protein synthesis. Such stabilizing molecules have been found in many bacterial species (e.g. the poly-purine sequences of Bacillus subtilis bacteriophage SP82).

Methodologies Tailored For Specific Therapeutic Targets

A. Genetic Therapy Approaches For Cancer

Gene therapy trials for treatment of cancer represent the major portion of research experimentation as well as the major contribution to advances in gene therapy techniques. A countless number of such techniques have been, and still being continually, designed aiming at targeting nearly all known pathogenetic mechanisms underlying development and progression of various aspects of the malignant phenotype.

The three conventional modalities of treatment of cancer, surgery radiotherapy and chemotherapy, are often unsuccessful in treating cancer. Gene therapy is the emerging fourth modality for treatment of cancer. It can be used either alone or as an adjuvant to other treatment modalities. Certain genes can sensitize tumor cells to radiation or drugs and hence can be used to enhance the effect of the treatment. Gene therapy can also be used to debulk tumors which can then be removed by surgery. Various approaches are being examined in clinical trials for gene therapy for cancer, they include :

I. Targeting genetic lesions in tumor cells by antisense molecules

Antisense molecules are synthetic oligo-deoxy-nucleotides which are designed such that they can hybridize specifically to the coding (sense) mRNA inside the cell. Targeting mRNA with these molecules is attractive as they form Watson-Crick base pairs with the targeted mRNA. The double stranded RNA cannot be translated and is easily destroyed with consequent arrest of synthesis of oncoproteins that mediate tumor development thus leading to halting of malignant progression, invasion and metastasis.

II. Immunomodulation by gene therapy

Cancer patients generally have lowered immune response which can be augmented by gene therapy. It is now possible to genetically alter immune cells to increase their function. Therapeutic genes can be introduced ex-vivo either into the tumor cells or into the effector cells such as T lymphocytes or antigen presenting dendritic cells, or even to proximal or distant organ sites in the patient. Such a strategy can be used in combination with other strategies or even with any conventional treatment modality of cancer.

Tumor cells as well as immune effector cells have been modified by insertion of various genes mainly cytokine and growth factor genes. Cytokines, involved in immunity and inflammation, are being extensively used in immunotherapy. Genetically modified tumor cells releasing cytokines have been shown to result in local recruitment of inflammatory cells that, in turn, effectively inhibit tumor growth. This is accompanied by tumor antigen priming of the host immune system and enhanced tumor immunogenicity resulting in tumor regression. In animal studies, in some instances immunological memory has been generated to resist subsequent challenge with unmodified parental tumor cells. In a selected set of advanced cancer patients it has been demonstrated that high dose of the cytokine, interleukin-2 (IL-2), results in modification of the host immune system leading to tumor regression.

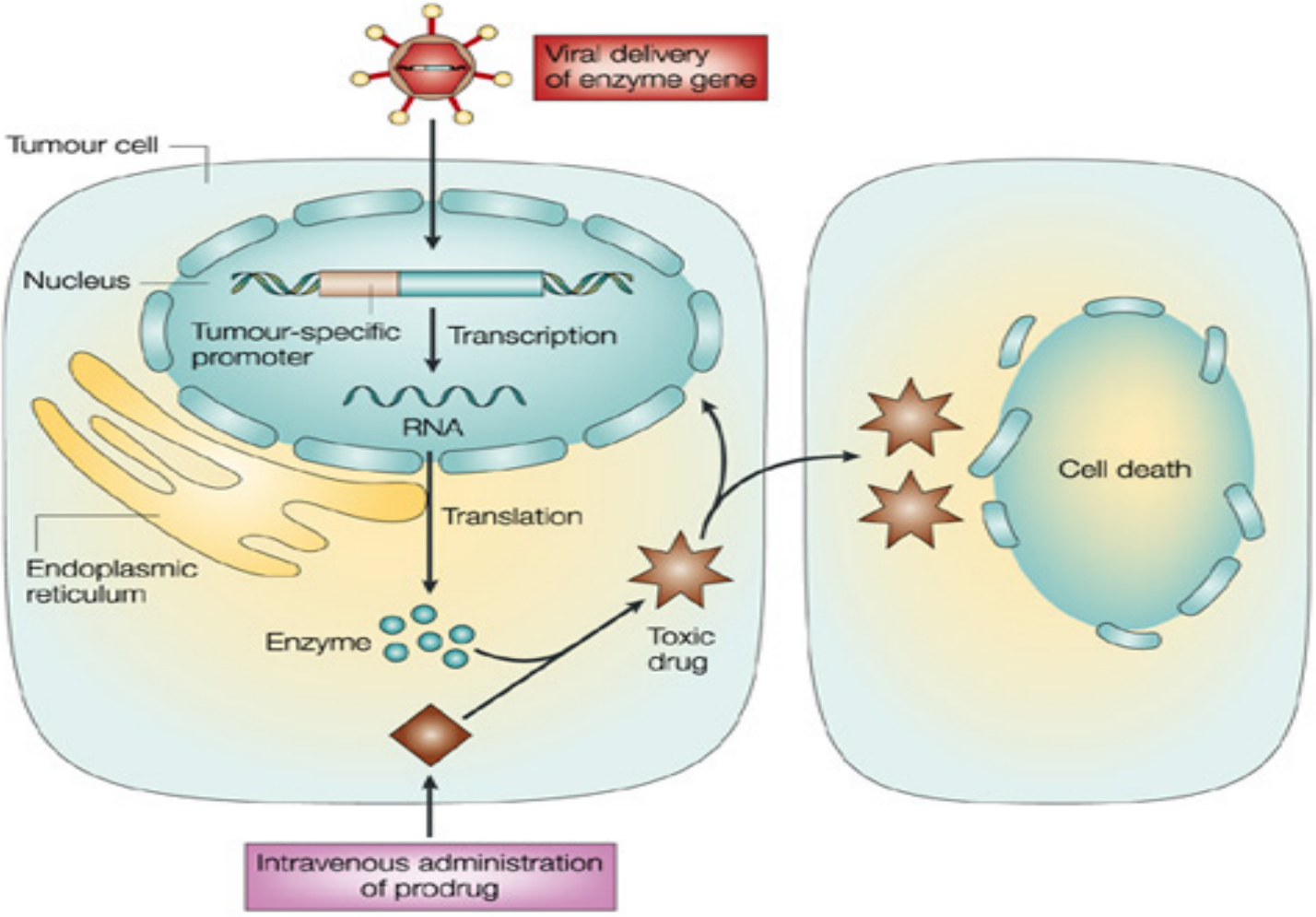
III. Induction of apoptosis

One of the major problems in treating solid tumors by either radiation therapy or chemotherapy is that the tumor cells are often resistant to apoptosis and therefore do not succumb to the conventional treatment. Hence, many therapeutic approaches have been aimed at killing cancer cells by inducing apoptosis. At the molecular level, mutation of the p53 tumor-suppressor gene is found in greater than 50% of human tumors. p53 plays a major role by inducing apoptosis in cells carrying damaged DNA. Wild type p53 has been shown to induce apoptosis in squamous cell carcinoma cell lines and has also been used in phase-1 trials of adenoviral-p53 transfer in patients with advanced squamous cell carcinoma in a surgical adjuvant setting. Wild type p53 has been used either alone or in combination with other apoptosis-inducing genes, or in combination with radiotherapy. Overexpression of pro-apoptotic molecules, e.g. Bax, favor death of cells resistant to ionizing radiation. Expression of Bax could sensitize radio-refractory cells to radiotherapy. Caspase-8, a member of the family of Caspases is also involved in bringing about apoptosis. Preclinical studies have indicated that caspase-8 effectively induced cell death in gliomas and could be a useful strategy for gene therapy of these tumors.

IV. Blocking of tumor angiogenesis

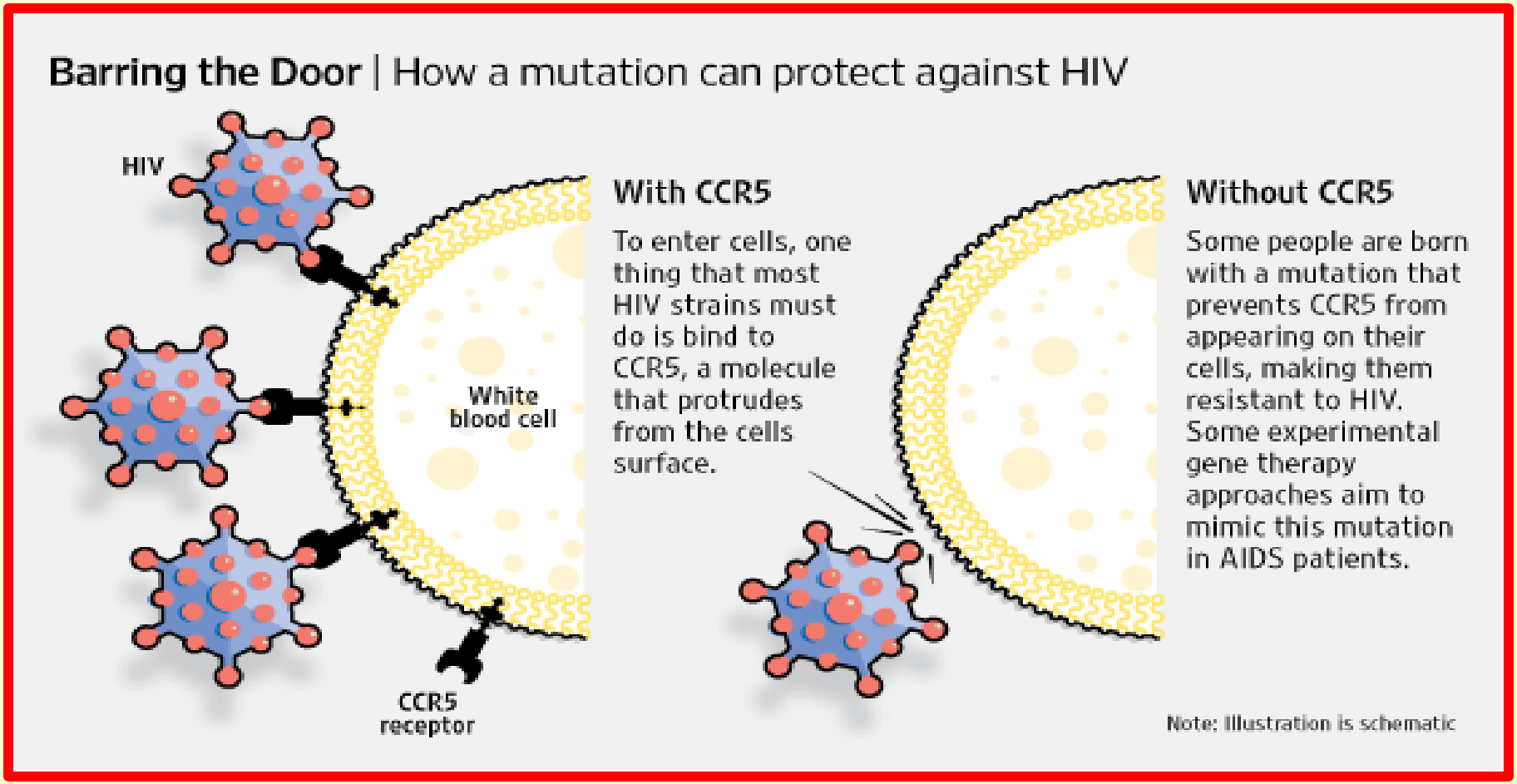
Tumors require a constant supply of oxygen, nutrients, hormones and growth factors for their existence and dissemination. This is provided by formation of new blood vessels or angiogenesis. Experimental tumors have been shown to regress by inhibiting angiogenesis and this has made it a suitable target for gene therapy. Two popular inhibitors of angiogenesis are angiostatin and endostatin. These are naturally generated by proteolysis of larger proteins such as plasminogen (for angiostatin) and collagen XVIII (for endostatin). Gene therapy trials are being conducted with human recombinant endostatin. Also, genes coding for the angiogenesis inhibitors can be introduced either directly into the patient's cells ex vivo or through generic cells that have been genetically modified to overexpress the protein of interest.

Drug Potentiation Approach In Cancer Therapy



6. Methodologies Tailored For Specific Therapeutic Targets

B. Induction Of Protective Mutations In Prophylaxis Against Infectious Diseases



CURRENT STATUS OF GENE THERAPY TRIALS

The Food and Drug Administration (FDA) has not yet approved any human gene therapy product for sale. Current gene therapy is largely experimental and has not proven very successful in clinical trials. Little progress has been made since the first gene therapy clinical trial began in 1990. In 1999, gene therapy suffered a major setback with the death of 18-year-old patient who was participating in a gene therapy trial for treatment of ornithine transcarboxylase deficiency (OTCD). The patient expired from multiple organ failures 4 days after starting the treatment. His death is believed to have been triggered by a severe immune response to the adenovirus carrier.

Another major blow came in January 2003, when the FDA placed a temporary halt on all gene therapy trials using retroviral vectors in blood stem cells. FDA took this action after a second child treated in a gene therapy trial had developed a leukemia-like condition. Both this child and another who had developed a similar condition in August 2002 had been successfully treated by gene therapy for X-linked severe combined immunodeficiency disease.

In spite o these disappointments, the prospects of genetic therapy for both genetic and non-genetic diseases are quite hopeful in view of the rapidly progressive increase in our knowledge about our genetic material. As we know more about the structure of our genome and have more better understanding of the functions of our genes, new precise treatment methodologies could be designed and tried and expectations of their ultimate success in treatment of concerned diseases could be a solid reality.

www.archive.org/details/MedicalGenetics

www.sites.google.com/site/mszsalemsite/

www.4shared-china.com/u/OheFbHEo/mszsalem.html